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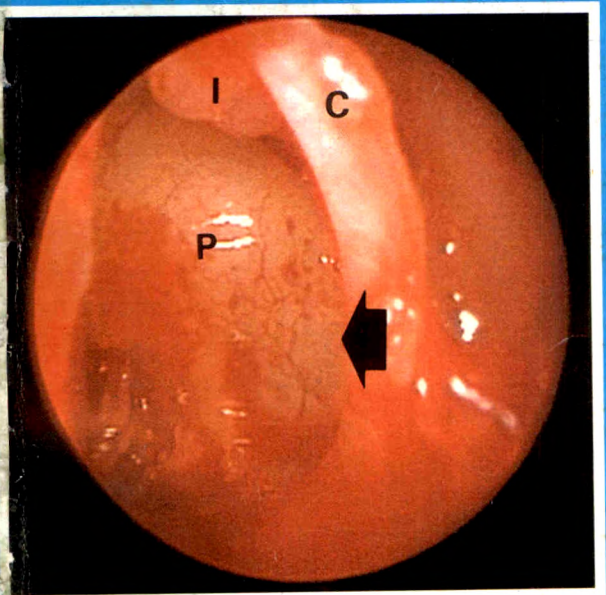
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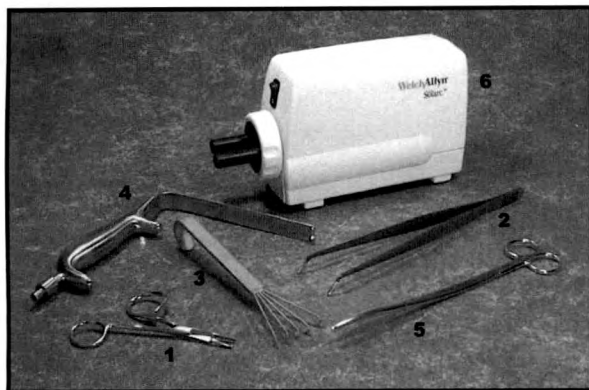
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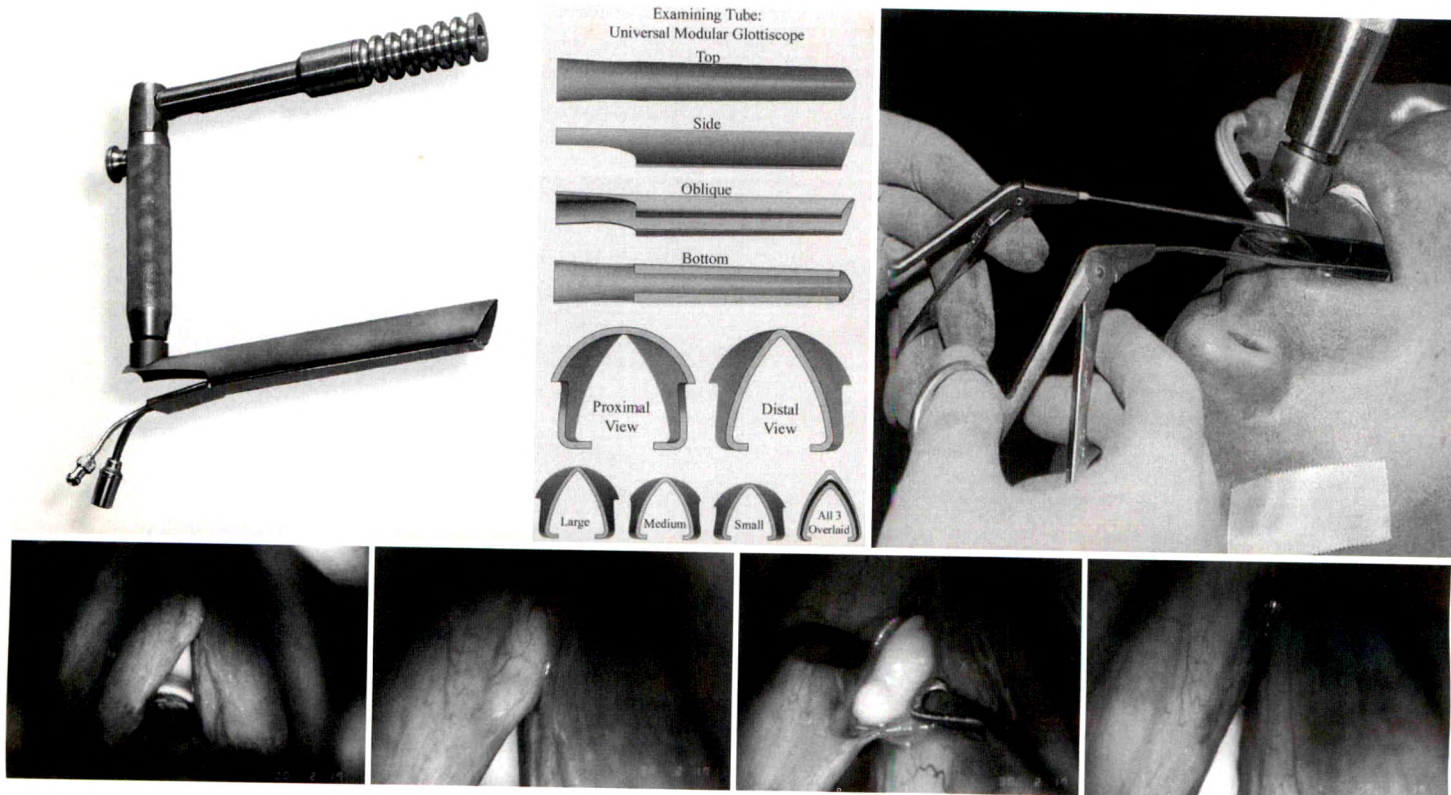
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FLUORODEOXYGLUCOSE IMAGING USING A COINCIDENCE GAMMA CAMERA TO DETECT HEAD AND NECK SQUAMOUS CELL CARCINOMA AND RESPONSE TO CHEMOTHERAPY

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This prospective study was performed to evaluate the ability of a dual-head gamma camera with fluorine-18 fluorodeoxyglucose coincidence detection emission tomography (FDG-CDET) to detect primary tumor and cervical lymph nodes in head and neck squamous cell carcinoma (HNSCC), and to show the response of the carcinoma to chemotherapy. The findings were compared with those of physical examination, computed tomography (CT), and histopathology, before treatment in 61 patients, and after induction chemotherapy in 34 of them. Before treatment, the primary was detected in 93%, 79%, and 95% of cases on panendoscopy, CT, and FDG-CDET, respectively. After chemotherapy, 34 patients were evaluable for response of the primary tumor. Surgical resection was performed in 23 of them: agreement with histopathologic results for response to treatment was 74%, 69%, and 78% for panendoscopy, CT, and FDG-CDET, respectively. No surgical resection was performed in 11 of the 34 patients, but biopsies were performed before radiotherapy, and their rates of agreement with histopathologic results for response to treatment were 75%, 75%, and 67% on panendoscopy, CT, and FDG-CDET, respectively. For cervical lymph nodes, 245 sites were resected in 41 patients, and FDG-CDET appeared competitive with CT in detecting metastatic neck disease, especially after neoadjuvant chemotherapy; the accuracy was 93%. These results demonstrated the ability of FDG-CDET to detect primary tumors and cervical lymph nodes in HNSCC and to show its response to chemotherapy, as compared to the ability of CT and panendoscopy. It may be a complementary tool to evaluate residual disease after induction chemotherapy, although higher sensitivity would be required for FDG-CDET to be considered as a staging modality.

KEY WORDS — chemotherapy, coincidence detection emission tomography, fluorine-18 fluorodeoxyglucose, head and neck carcinoma, positron emission tomography.

INTRODUCTION

Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) is now used to detect a variety of malignancies, based on the high rate of fluorine-18 fluorodeoxyglucose (FDG) uptake by malignant tissues.¹ In head and neck tumors, uses and limitations of FDG-PET have been demonstrated in detecting primary tumors, cervical lymph node and distant metastases, and recurrences and in identification of unknown primary tumors.²⁻¹⁶

Induction chemotherapy is used in cases of advanced malignancies of the head and neck before either radiotherapy or surgery.^{17,18} Assessment of the effect of chemotherapy and evaluation of residual tumor in responders are essential for planning further therapy,¹⁹ but no conventional method is reliable for assessing the response to chemotherapy, insofar as the response may be discordant between the primary and the nodes. Since FDG-PET is a functional imaging modality in tumors, through assess-

ment of glucose uptake, it could provide useful data for evaluating the response of head and neck squamous cell carcinoma (HNSCC) to therapy — especially the early effects of neoadjuvant chemotherapy.²⁰⁻²² In addition, the need to perform unilateral or bilateral neck dissection in cases staged as N0 could be evaluated, given the reported sensitivity rate of FDG scanning, since no conventional diagnostic modalities are reliable enough to detect cervical lymph node involvement before operation.

However, the availability of dedicated PET machines is restricted. The conventional dual-head gamma camera of our Department of Nuclear Medicine has been designed to image FDG by coincidence detection of the two 511-keV photons resulting from each disintegration of this radiopharmaceutical.

Although the diagnostic value of FDG-PET in HNSCC for detecting primary tumors and cervical lymph node involvement has been well demonstrated, the value of FDG coincidence detection emission to-

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mography (FDG-CDET) is not well documented in large series, especially with respect to the variety of primary sites involved in HNSCC.^{14,23-25}

The purpose of this study was to prospectively evaluate the ability of FDG-CDET to detect primary tumors and metastatic neck disease, as well as the response to neoadjuvant chemotherapy, as compared to the ability of physical and endoscopic examinations and computed tomography (CT). All results were correlated with the histopathologic results for a comparative analysis.

PATIENTS AND METHODS

Sixty-one patients consecutively referred to our institution with previously untreated HNSCC were included in a prospective study. At first, 40 patients were enrolled in a clinical trial approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale). Subsequently, 21 patients had an examination after approval by the Agence Française du Médicament. Plasma glucose levels were used to exclude diabetic patients. Fifty-six patients were male, 5 were female, and their mean age was 57.9 years (range, 36 to 79 years). In addition to physical examination done on the patient awake and panendoscopy done under anesthesia, chest radiography, a CT scan (using a Philips Tomoscan 7000 helical CT scanner), and a FDG-CDET scan were performed within an interval of 2 weeks. The FDG-CDET and CT scans were done before endoscopy, and images were read independently, with only information about the physical examination performed on the patient awake. For the localization of cervical lymph nodes (detected by physical, CT, and FDG-CDET examinations and by histopathology), the classification based on the standardized neck dissection terminology (levels I to VI) was adopted.²⁶ In patients treated with induction chemotherapy, all of the examinations were performed before treatment and were repeated between 2 and 3 weeks after the last course of chemotherapy.

The first group of 17 patients (group 1) did not receive chemotherapy. Induction chemotherapy was performed in the second group of 44 patients (group 2), and consisted of a modification of the regimen of Al Sarraf et al,¹⁷ with cisplatin (20 to 25 mg/m² area per day) and 5-fluorouracil (1,000 mg/m² area per day) by continuous infusion for 4 days, on days 1 and 21. A third course of chemotherapy was performed on day 42 in responder patients (ie, a clinical response rate of more than 50%). The responses to chemotherapy of the primary tumor and cervical lymph nodes were evaluated by both clinical examination on the patient awake and panendoscopy under anesthesia, and by results of CT and FDG-CDET.

The FDG-CDET was performed with a gamma camera that was a Picker Prism 2000XP equipped with thick detection crystals and the coincidence detection system. Filters and septa are placed over the two detectors in order to get a 2-dimensional acquisition. The FDG-CDET examination started 45 minutes after the patient, who had fasted for 6 hours or more, was injected with 150 to 250 MBq of FDG (Flucis, supplied by Cis Bio International, Saclay, France). One tomographic acquisition of the head and neck, and one image of the whole body were obtained by scanning without attenuation correction by an external source. The duration of each acquisition was approximately 40 minutes. The tomographic images were reconstructed with an iterative algorithm and displayed on a computer either as slices in 3 orthogonal planes or by 3-dimensional volume rendering. A FDG-CDET scan was qualitatively considered positive for malignancy when foci of a higher rate of FDG uptake were observed as compared with the activity of normal muscular or lymphoid tissues and salivary glands. Two nuclear medicine physicians interpreted the FDG-CDET images without information about the CT scan, and consensus was obtained after discussion in cases of discordance.

With respect to the primary tumor and cervical lymph node involvement, prospective results obtained from the physical, panendoscopic, CT, and FDG-CDET examinations were correlated with the histopathologic findings from surgical specimens or biopsies. For cervical lymph nodes, each level was analyzed and the size of the lymph node involvement was measured on histopathologic examination. Histopathologic findings were considered as the standard diagnostic references, for a comparative analysis. Results were scored as pathological for the presence of viable primary tumor or viable cervical lymph node involvement (ie, on endoscopic and physical examinations, presence of a visible primary tumor and/or involvement of anatomic lymph nodes; on CT, presence of an infiltrating mass with contrast enhancement and/or lymph nodes with a diameter greater than 12 mm or central necrosis and ring enhancement) or nonpathological (no viable primary tumor and no viable cervical lymph node involvement) for the endoscopy and imaging modalities. The same score was considered after induction chemotherapy. The FDG estimation of response to chemotherapy was done as described by Lowe et al²²; the physicians were given images in a before-and-after comparison format. All images were displayed on a gray scale set at the same arbitrary maximum pixel value. The readers rated the finding for each patient as progression of the tumor (or lymph node involvement), no change in the tumor, decrease in metabolism of FDG but

TABLE 1. DETECTION OF PRIMARY TUMOR IN 17 PATIENTS WITH NO NEOADJUVANT CHEMOTHERAPY (GROUP 1)

Method	Sample Size	Tumor Identified	Tumor Not Identified
Panendoscopy	17	13	4
CT	17	11	6
FDG-CDET	17	15	2
Final histopathologic result*	17	16	1

CT — computed tomography; FDG-CDET — fluorine-18 fluorodeoxyglucose coincidence detection emission tomography.
*Based on biopsy or surgical specimen.

residual tumor present, or resolution of tumor. Only resolution of tumor (or lymph node involvement) was considered a nonpathological finding.

The sensitivity, specificity, predictive positive value, negative value, and accuracy for each examination were calculated. The sensitivity and specificity for the endoscopy and imaging modalities were compared by means of Fisher's exact test. Statistical significance was assumed when $p \leq .05$.

RESULTS

Primary Tumor. On the basis of the physical examination during panendoscopy and the further findings of CT, 4 cases were staged as T0 (exhibiting cervical metastasis from an unknown primary), 5 cases as T1, 29 as T2 (1 of these patients exhibited 2 synchronous primary tumors staged as T2), 15 as T3, and 8 as T4.

Among group 1, the 17 patients who did not receive induction chemotherapy, 4 patients had cervical lymph node metastases from an unknown origin. The stages in this group were T0 in 4 patients, T1 in 3 patients, T2 in 8 patients, T3 in 1 patient, and T4 in 1 patient. Surgical resection of the primary tumor was performed in 11 patients, whereas 6 patients were treated for their primary tumor with radiotherapy only. The primary tumor was accurately localized in 13, 11, and 15 patients (ie, 76%, 65%, and 88%) on panendoscopic examination, CT, and FDG-CDET, respectively (Table 1). In contrast to panendoscopic examination and CT, FDG-CDET located the primary unknown tumor in 3 of 4 patients in whom confir-

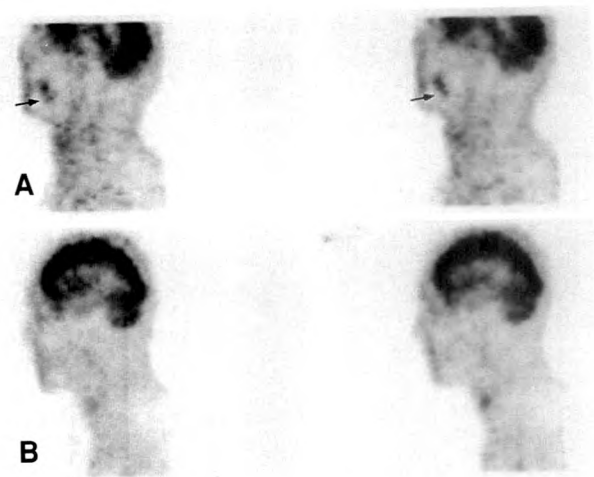


Fig 1. Fluorine-18 fluorodeoxyglucose coincidence detection emission tomography (FDG-CDET) images of patient showing **A**) fluorine-18 fluorodeoxyglucose (FDG) accumulation on oral cavity primary tumor (arrows) that **B**) disappeared after chemotherapy. Histopathologic results of surgical resection demonstrated residual primary tumor of 4 mm (false negative on FDG-CDET), and no cervical lymph node involvement in bilateral neck dissection (9 true-negative sites on FDG-CDET). Increased FDG activity at glottic area as observed is well known to occur secondary to FDG uptake by vocal muscles from speech activity.

matory biopsies were later performed: in the oropharynx, in the hypopharynx, and in the larynx. However, FDG-CDET missed 1 epiglottic tumor that was visualized on endoscopic examination and correctly identified by CT: histopathologic examination of this epiglottic resection showed a tumor measuring less than 4 mm. The results of both CT and FDG-CDET led to identification of all of the 16 primary tumors. Finally, the primary tumor was located in the oropharynx in 6 patients, in the larynx in 6, and in the hypopharynx in 4.

Among group 2, consisting of 44 patients, 1 patient exhibited 2 primary tumors, for a total of 45 tumors. The stages in this group were T1 in 2 patients, T2 in 22, T3 in 14, and T4 in 7. Before induction chemotherapy, the primary tumor was localized in 45, 38, and 44 cases (ie, 100%, 84%, and 98%) on panendoscopic examination, CT, and FDG-CDET, respectively (Table 2 and Figs 1 and 2). The primary tumor

TABLE 2. DETECTION OF PRIMARY TUMOR IN 44 PATIENTS (45 TUMORS) BEFORE NEOADJUVANT CHEMOTHERAPY COMPARED WITH HISTOPATHOLOGIC RESULTS OF BIOPSIES (GROUP 2)

Method	Cases	T+	T-	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Endoscopy	45	45	0	45	0	0	0	100	100	N/A	100	N/A
CT	45	40	7	38	0	2	7	84	94	N/A	95	N/A
FDG-CDET	45	44	1	44	0	0	1	98	98	N/A	100	N/A

Results were scored as pathological (T+; presence of primary tumor) or nonpathological (T-; no primary tumor) for endoscopy, CT, and FDG-CDET and compared with reference to histopathologic results of biopsies (45 positives).

TP — true positive; TN — true negative; FP — false positive; FN — false negative; PPV — positive predictive value; NPV — negative predictive value; N/A — not applicable.

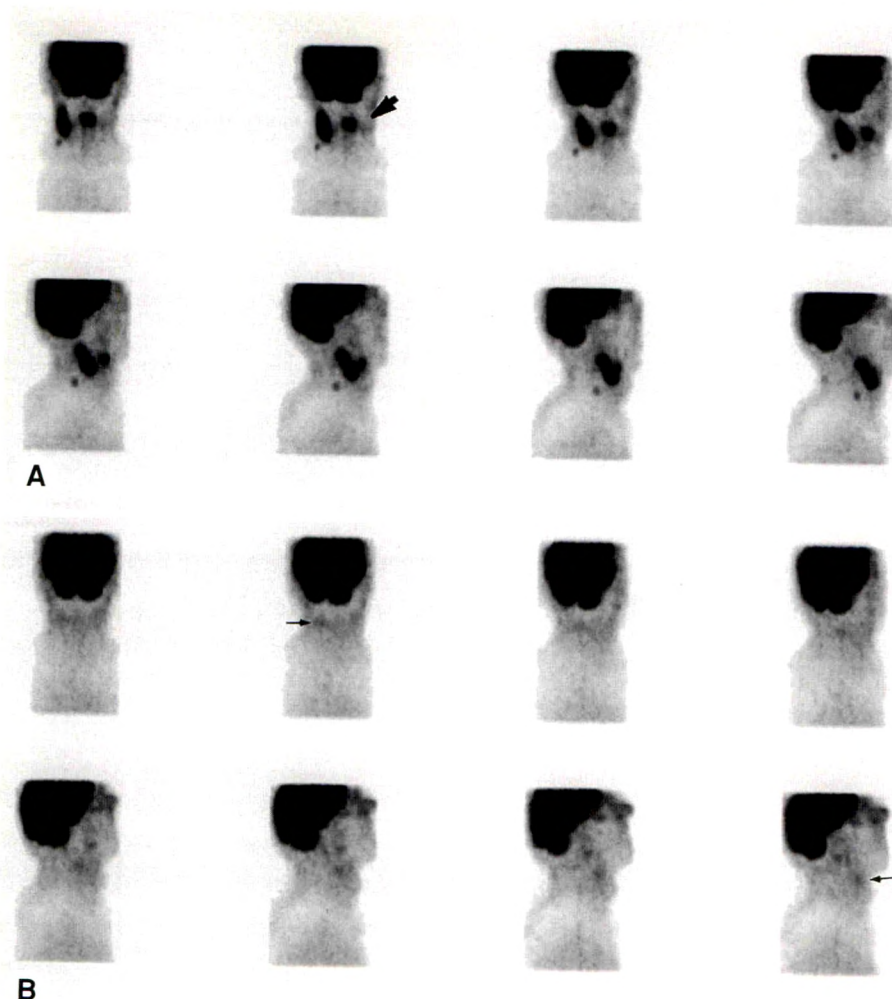


Fig 2. FDG-CDET images of patient showing **A**) intense FDG accumulation on vallecule primary tumor (arrowhead) that **B**) disappeared after chemotherapy. Two right cervical lymph nodes (levels II and III) were also observed before chemotherapy, but only 1 abnormal area of apparent FDG accumulation (level II) persisted after induction chemotherapy (arrows). Histopathologic results of surgical resection demonstrated no residual primary tumor (true negative on FDG-CDET), and 1 right cervical lymph node measuring about 7 mm in bilateral neck dissection (1 true-positive site and 7 true-negative sites on FDG-CDET).

was located in the mouth in 5 patients, in the rhinopharynx in 1 patient, in the oropharynx in 12 patients, in the larynx in 18 patients, and in the hypopharynx in 9 patients. Like CT, FDG-CDET missed 1 tumor of the lateral part of the tongue. Computed tomography was false-positive in 2 cases. All of the true-positive cases on CT were detected by FDG-CDET. The superior sensitivity of FDG-CDET in comparison with CT in detecting primary tumors was very close to statistical significance ($p = .058$).

Overall, in groups 1 and 2, the primary tumor was localized in 93%, 79%, and 95% of cases on panendoscopy, CT, and FDG-CDET, respectively.

Thirty-four group 2 patients (ie, 35 primary tu-

mors) were evaluable for full assessment after completion of chemotherapy; the examinations (panendoscopic examination, CT, and FDG-CDET) required to evaluate the response were all performed. The 10 other patients were excluded because these examinations were not performed in the specified time.

Surgical resection of the primary tumor was performed in 23 patients of this group (ie, 23 primary tumors; Table 3). Histopathologic findings from surgical specimens were positive for persistence of viable cancer in 16 patients (ie, 16 primary tumors), and negative in 7 patients (ie, 7 primary tumors). Overall accordance with histopathologic results was observed in 17 (74%), 16 (69%), and 18 (78%) pa-

TABLE 3. DETECTION OF RESIDUAL PRIMARY TUMOR AFTER CHEMOTHERAPY IN 23 PATIENTS (23 TUMORS) COMPARED WITH HISTOPATHOLOGIC RESULTS OF SURGICAL SPECIMENS (GROUP 2)

Method	Cases	T+	T-	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Endoscopy	23	18	5	14	3	4	2	74	87	43	78	60
CT	23	13	10	11	5	2	5	69	69	71	85	50
FDG-CDET	23	13	10	12	6	1	4	78	75	86	92	60

Results were scored as pathological (T+: presence of primary tumor) or nonpathological (T-: no primary tumor) for endoscopy, CT, and FDG-CDET, and compared with reference to histopathologic results of surgical specimens (16 positive tumors, 7 negatives).

TABLE 4. DETECTION OF RESIDUAL PRIMARY TUMOR AFTER CHEMOTHERAPY IN 11 PATIENTS (12 TUMORS) COMPARED WITH HISTOPATHOLOGIC RESULTS OF BIOPSIES (GROUP 2)

Method	Cases	T+	T-	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Endoscopy	12	7	5	4	5	3	0	75	100	62	57	100
CT	12	3	9	2	7	1	2	75	50	87	66	78
FDG-CDET	12	4	8	2	6	2	2	67	50	75	50	75

Results were scored as pathological (T+; presence of primary tumor) or nonpathological (T-; no primary tumor) for endoscopy, CT, and FDG-CDET, and compared with reference to histopathologic results of biopsies performed after chemotherapy (4 positives).

tients on panendoscopic examination, CT, and FDG-CDET, respectively. At FDG-CDET, there were 12 true-positive and 6 true-negative cases (Fig 2). Two of the 4 false-negative cases in FDG-CDET were malignant residual tumors measuring less than 5 mm (also not detected on CT; Fig 1). The false-positive case in FDG-CDET was an oropharyngeal tumor exhibiting residual focus of FDG uptake, which was interpreted as positive (in contrast to CT). Concerning sensitivity, no difference between the modalities reached the level of statistical significance ($p > .26$). The only significant value was the higher specificity of FDG-CDET in comparison to endoscopy.

Eleven patients (12 primary tumors) were treated with radiotherapy after induction chemotherapy (Table 4). Biopsies were positive for cancer in 4 cases before radiotherapy. Accordance with biopsy results was observed in 9, 9, and 8 cases on panendoscopy, CT, and FDG-CDET, respectively.

Lymph Node Involvement. Histopathologic results of neck dissection procedures were compared to those of physical examination, CT, and FDG-CDET in 41 patients (from groups 1 and 2) who were operated on (21 cases were N0, 4 were N1, 8 were N2a, and 8 were N3 according to physical examination; Table 5). Induction chemotherapy was performed before neck dissection in 28 patients (from group 2). In these

28 patients, histopathologic results of neck dissections were compared to physical examination, CT, and FDG-CDET performed after induction chemotherapy. A radical, modified radical, or selective neck dissection (unilateral or bilateral) was performed. A total of 245 lymph node sites (from levels I to VI, according to the standardized neck dissection terminology²⁶) were resected and evaluated. In 24 patients, the histopathologic findings were positive for malignancy in 45 lymph node sites. The size of the cervical lymph node involvement measured on histopathologic examination ranged from 1.8 to 50 mm (Fig 2). Overall, in both groups, accordance with histopathologic results was observed in 219 (89%), 224 (91%), and 227 (93%) lymph node sites on physical examination, CT, and FDG-CDET, respectively. The sensitivity was less than 61% for all examinations, and the other values (specificity, positive predictive value, and negative predictive value) were not significantly different from each other. All cases with false-negative FDG-CDET findings were either cervical metastases with a diameter of less than 7.8 mm (1.8 to 7.8 mm; Fig 1) — one of them being, however, detected by CT — or had a lack of anatomic detail that led us to group together two separate lymph nodes in a single site. In the group of 28 patients who received neoadjuvant chemotherapy before neck dissection, the accuracy of FDG-CDET reached 93%, and that of

TABLE 5. DETECTION OF CERVICAL SITE METASTASES IN 41 PATIENTS (GROUPS 1 AND 2) COMPARED WITH HISTOPATHOLOGIC RESULTS OF NECK DISSECTIONS (45 POSITIVE SITES INVOLVED IN 245 SITES RESECTED)

Method	Sites	N+	N-	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Physical												
No AC	77	10	67	9	59	1	8	88	53	98	90	88
AC	168	13	155	12	139	1	16	90	43	99	92	90
Both	245	23	222	21	198	2	24	89	47	99	91	89
CT												
No AC	77	12	65	11	59	1	6	91	65	98	92	91
AC	168	18	150	16	138	2	12	92	57	98	89	92
Both	245	30	215	27	197	3	18	91	60	98	90	92
FDG-CDET												
No AC	77	10	67	10	60	0	7	91	59	100	100	89
AC	168	17	151	17	140	0	11	93	61	100	100	93
Both	245	27	218	27	200	0	18	93	60	100	100	92

Results were scored as pathological (N+; cervical lymph node involvement) or nonpathological (N-; no cervical lymph node involvement) for physical examination, CT, and FDG-CDET, and compared with reference to histopathologic results of neck dissections, in patients without neoadjuvant chemotherapy, in patients with neoadjuvant chemotherapy, and in both of groups of patients.

Physical — physical examination; AC — adjuvant chemotherapy.

CT, 92%. The sensitivities and specificities of CT and CDET were not statistically different ($p > .24$).

Distant Metastatic Involvement. The chest radiograph was suspicious for metastases in 5 patients. Eight patients showed distant metastases on FDG-CDET: in the lung in 6 patients, in the mediastinum in 1 patient, and in the hip in 1 other. Computed tomography was consequently performed and identified metastatic disease in 6 cases (6 in the lung and 1 in the hip). Four of these metastases were proven histologically, and 3 patients eventually died. In the remaining case (mediastinal metastasis), the lesion disappeared on both CT and FDG-CDET after adjuvant chemotherapy, and biopsy was then negative.

DISCUSSION

While panendoscopy and conventional imaging (CT and magnetic resonance imaging) are the standard means for detecting malignancy, FDG scanning is a new procedure in the evaluation of head and neck carcinomas. It is a noninvasive functional imaging modality that assesses glucose uptake by malignancies. Its clinical utility has been demonstrated in detection of primary tumors, recurrences, and cervical nodes and distant metastases in HNSCC.²⁻¹⁶ Most studies have used dedicated PET cameras. However, CDET cameras, more available than PET, seem to have resolution that is nearly as good as that of PET cameras.²⁷ The utility of FDG-CDET has been previously reported in HNSCC of the oral cavity and oropharynx, in detection of unknown primary tumor and of recurrent disease and residual tumor after therapy.^{14,23-25} Another promising use of FDG imaging may be its ability to measure the effect of induction chemotherapy, especially in responders, to guide further adequate therapy. This present study was performed to evaluate whether FDG-CDET will be as effective as FDG-PET in detecting primary tumors of HNSCC at various sites, as well as cervical node metastases, and also to study its ability to evaluate responses to induction chemotherapy.

This present study demonstrated that FDG-CDET was successful in detecting, before therapy, the primary tumor in the different sites involved in HNSCC (oral cavity, oropharynx, laryngeal, and hypopharyngeal regions). It appeared to be more reliable than CT (the sensitivity of FDG-CDET being very close to reaching statistical significance), and in patients with unknown primary tumors, it was successful in detecting the primary site. This ability of FDG scanning to detect occult primary tumors of the head and neck has been demonstrated with dedicated PET cameras^{8,11,12} or FDG-SPECT (single photon emission tomography)^{28,29} and also CDET cameras.^{14,24} In our series of 61 tumors (16 in the first group and 45 in

the second group before chemotherapy), FDG-CDET correctly detected all primary sites except 2: 1 of the epiglottis and 1 of the tongue. The tumor of the epiglottis was detected on CT, so that the results were complementary between CT and FDG-CDET in the primary detection. However, the size of the epiglottis tumor — less than 4 mm on histopathology — probably made it too small to be detected by FDG-CDET. Usually, FDG scanning is able to detect lesions as small as 6 mm^{6,27}; an in vivo resolution of 6 to 8 mm appears to be the limit of the technique. In contrast, the asymmetry of the epiglottis, more than the tumor itself, probably led to a true-positive CT for the primary site. The second tumor missed on FDG-CDET was a T1 tumor of the lateral tongue that was not detected because an increased activity of FDG in the saliva pools caused artifacts. No false-positive findings were observed in the population before therapy, partly because FDG imaging was performed before biopsy to avoid inflammation and nonspecific increased FDG uptake.⁶ This is a point of paramount importance in the routine use of FDG scanning to avoid false-positive results. In the literature, the sensitivity of FDG scanning in detecting the primary tumor in HNSCC with dedicated PET cameras ranges from 88% to 100%.^{2-6,10,16,30} The rate reached 100% with a dual-head coincidence camera in a prospective report that included 18 untreated patients, and the specificity was 90%.²³ Our results could not be compared to those of Pai et al²⁴ (sensitivity of 71%), since that series reported 7 patients with unknown primaries that included HNSCC but also other tumors. The results of the present prospective study, in a larger number of patients, are consequently in agreement with those of Stokkel et al,²³ demonstrating that CDET gamma cameras have, in this application, performance similar to that of dedicated PET cameras.

The utility of FDG scanning after therapy, especially in detecting recurrent or residual disease after radiotherapy, has been demonstrated.^{6,8,10,15,21,24,25,30} In contrast, only small series have evaluated PET scanning as a measure of response to chemotherapy in HNSCC, based on the quantitative change in FDG uptake.²⁰⁻²² Neoadjuvant chemotherapy may be used to increase the possibilities of larynx preservation and conservative surgery.^{18,19,31} However, no clinical, biological, or imaging modalities are currently available to predict chemotherapeutic response. Reisser et al²⁰ demonstrated that FDG-PET provides early quantitative data on tumor metabolism before and after chemotherapy. Lowe et al,²² studying the response to neoadjuvant chemotherapy in 28 patients in an organ preservation protocol, with HNSCC staged T3 to T4, found a sensitivity for FDG-PET of 90% and a specificity of 83%, as compared to re-

sults of tissue biopsies. These authors also showed that visual evaluation, which was used in the present study, was at least as efficient as semiquantification (ie, standardized uptake value or SUV measurements), which would have led to 4 false-positive results without correcting the 2 false-negative cases in their series. In another study, FDG-PET also appeared useful in assessing the response to chemotherapy of the primary tumor, which was evaluated by biopsies.³² However, the performance has not yet been evaluated on CDET machines, which are not currently well suited for quantification (ie, SUV). In our study, among 23 surgical resections of the primary tumor, the accuracy, with histopathologic results as the reference, was the highest for FDG-CDET (78%), as compared to 74% for panendoscopic and 69% for CT examinations, but the difference was not significant. In contrast, the superior specificity of FDG-CDET in comparison to endoscopy reached the level of statistical significance, and the false-positive case was an oropharyngeal tumor exhibiting only residual focus of FDG uptake. Two of the 4 false-negative cases of FDG were residual primary malignant tumors measuring less than 5 mm, and the 2 others were tumors of the lateral tongue (including 1 missed previously before chemotherapy). In the population of patients who had no surgical resection of their primary tumor (12 primary tumors in 11 patients), histopathologic evaluation of chemotherapy efficacy was only evaluated on tissue biopsy specimens taken during endoscopy. The accuracy of FDG-CDET was only 67%, with no statistical significance in comparison to other modalities. However, biopsies have a lower confidence value than surgical resection in evaluating residual tumor and response to chemotherapy.³³ As a confirmation of that point, in the group of 23 patients operated on after chemotherapy, 20% of the biopsies of the primary tumor performed after completion of chemotherapy were false-negative as compared to the surgical specimen. The accuracy of different modalities in evaluating residual tumor after induction chemotherapy cannot consequently be determined from these data, since histopathologic findings from biopsies may not be considered as a reference.

Concerning the detection of cervical lymph nodes, involvement could only be accurately evaluated after surgery and in correlation with histopathologic findings of neck dissections, and the results therefore include patients who received induction chemotherapy before the surgical procedure. The overall accuracy of FDG-CDET (93%) was competitive with that of CT (91%). The sensitivity of FDG-CDET was similar to that of CT, and its specificity was 100% (98% for CT), since no false-positive cases were ob-

served. All false-negative cases on FDG-CDET, with or without induction chemotherapy, were either cervical metastases with a diameter of less than 7.8 mm or were due to a lack of anatomic detail that led us to group together two separate lymph nodes in a single site. For example, it was difficult to distinguish whether two foci suspected to be malignant cervical lymph nodes on FDG-CDET were located in a single level or in two adjacent levels of the neck. This sensitivity rate was not as good as that in the study of Stokkel et al,²³ in which the sensitivity was 89% (and the specificity 93%). However, these authors only took into consideration the neck side involved, and not the number of cervical lymph node sites, while comparing the results with the histopathologic findings.²³ With FDG-PET machines, previous studies have demonstrated sensitivity and specificity in detecting cervical metastases to be in the ranges of 67% to 100% and 82% to 100%, respectively.^{5,7-9,16,34,35} A sensitivity of 50% was also reported by Paulus et al.¹⁰ This wide range of results is probably due to the different methods of analysis used. In the present study, a resolution of 7 to 8 mm in diameter appeared to be the limit of the FDG-CDET for detecting cervical metastases.

We conclude that FDG-CDET may be a useful tool for evaluating viable cervical lymph node metastases after neoadjuvant chemotherapy. Moreover, the responses of the cervical nodes, assessed separately, did not determine the choice of treatment for the primary tumor. No other study, to our knowledge, has correlated histopathologic findings of neck dissection to the results of FDG-CDET after courses of chemotherapy. The results of FDG-CDET in evaluating the response were, in this study, better than those of physical examination, but were, however, similar to those of CT not statistically different. In view of these results, the sensitivity of FDG-CDET appeared to be too low to select patients in whom neck dissection could be spared in cases in the N0 stage, especially when organ preservation is required. Staging N0 patients as N+ or N- (with or without histopathologic lymph node involvement) from neck dissections remains a point of great interest in discussing the necessity of complementary postoperative radiotherapy, especially when tumor-negative margins are observed on the resected primary. In contrast to surgical deflation, which we perform in selected cases on the primary tumor after induction chemotherapy,¹⁹ only an increased sensitivity of FDG-CDET could allow surgical deflation of the cervical lymph nodes.

In the detection of distant metastases, FDG-CDET led to identification of 3 metastases that were not visualized during the routine preoperative examinations

(chest radiography). In 1 case, however, the suspected metastasis was not proven histologically, but it had disappeared on CT and FDG-CDET after chemotherapy, and the patient is still alive with no sign of disease 2 years later. In the 2 other cases not proven histologically, the patients died of lung metastases. A previous study using FDG-PET reported a sensitivity of 90% and a specificity of 94% for the detection of distant metastases.³⁶ Like FDG-PET, FDG-CDET could also be useful in detecting distant metastases at the whole-body level and in choosing appropriate therapeutic modalities. However, no statistical study was done, since histopathologic findings, considered to be the reference in this present study, were not available or were negative, and since the number of distant metastases was too low to make valuable comparisons.

CONCLUSION

In conclusion, FDG-CDET, like FDG-PET per-

formed with dedicated PET machines, was useful in detecting primary malignancies and cervical and distant metastases in HNSCC. Based on the metabolic activity of the tumor and metastases, it may also be helpful in evaluating the response to neoadjuvant chemotherapy. It could be included in diagnosis of HNSCC, more as a complementary tool than as an alternative imaging modality, since its results were not statistically significantly different from those of other modalities (CT and results of physical and endoscopic examinations); FDG-CDET may be more specific than CT, because of the limits of its resolution, but it is not more sensitive. However, the superior sensitivity of FDG-CDET as compared with CT in detecting primary tumors before treatment was very close to significant. Higher resolution of images with improvement of CDET by emergent techniques such as attenuation correction by an external source and the 3-head coincidence gamma camera could increase the sensitivity of FDG-CDET, with potential therapeutic implications.

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MANAGEMENT OF CAROTID ARTERY INVASION IN ADVANCED MALIGNANCIES OF HEAD AND NECK

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The objective of this study was to retrospectively investigate a single institution's experience with carotid artery resection performed as part of an oncological procedure and to determine acute and convalescent complication and survival rates. We performed a record review of 28 patients with head and neck malignancy invading the carotid artery. Immediate carotid artery resection and ligation on an emergent basis was performed on 12 patients (group 1), elective resection and ligation was performed on 8 patients (group 2), and elective resection and revascularization was performed on 8 patients (group 3). In group 1, although 1 patient survived for 1 year and 1 patient survived for 2 years, 1 patient died of severe neurologic deficit, 2 patients experienced neurologic deficit with good recovery, and 1 patient was moderately disabled. In group 2, 2 patients survived without disease for 5 years, and 2 patients experienced neurologic deficit, 1 with good recovery and the other with complete recovery. In group 3, only 1 patient survived for 5 years, and within this group, 1 patient died of severe neurologic deficit, 1 patient had neurologic deficit with moderate recovery, and 1 patient had neurologic deficit with complete recovery. No significant difference in mortality and morbidity rate was observed between the "resection and ligation" group and the "resection and revascularization" group ($p = .52$, $\chi^2 = 0.79$). We conclude that the surgical treatment of patients with an invaded carotid artery, including carotid resection, provides a small but real chance of 5-year survival. The methods of carotid resection and repair should be guided by clinical presentation and by preoperative and intraoperative investigations.

KEY WORDS — carotid artery invasion, head and neck malignancy.

INTRODUCTION

Carotid artery invasion, regarded as a criterion for inoperability by some investigators,^{1,2} is a sign of unfavorable prognosis in patients with advanced head and neck malignancy. In such instances, if the patient is left without treatment or receives nonsurgical palliative therapies or nononcological surgical treatment, the chance of survival for the patient becomes almost zero. Various authors in the literature have emphasized this fact by indicating that very few of these patients were long-term disease-free survivors.³ The only treatment option that offers cure for the patient is the radical extirpation of the invaded carotid artery system.

Despite the possibility of providing local tumor control, there are many different opinions among researchers about this major surgical procedure that carries such a high perioperative morbidity rate and such a disputable increase in survival rate. The discussion is not limited to whether to operate, but also involves the issue of revascularization after resection and its methods. Some investigators^{1,2,4} prefer revascularization after resection, whereas others⁵⁻⁷ are in favor of resection without revascularization. These methods

vary greatly with regard to morbidity and mortality rates. The differences in the mortality and morbidity rates may be a result of the small patient populations used in these studies (Table 1^{1,2,4-11}). Aside from a meta-analysis,⁹ the number of patients in these studies is relatively small. Unfortunately, the studies that compare all surgical treatment methods are extremely rare.

TABLE 1. PUBLICATIONS RELATED TO CAROTID ARTERY INVASION IN ADVANCED HEAD AND NECK MALIGNANCIES

Authors	Year	No. of Patients
Biller et al ²	1988	26
de Vries et al ⁶	1988	8
Yazicioglu et al ⁸	1989	10
Maves et al ⁷	1992	20
Snyderman and D'Amico ⁹	1992	158*
Jacobs et al ¹⁰	1994	11
Meleca and Marks ⁴	1994	20
Brennan and Jafek ⁵	1994	7
Okamoto et al ¹¹	1996	24
Katsuno et al ¹	1997	7

*Meta-analysis (total of 158 patients in 22 separate published series).

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In this report, we analyze retrospectively all surgical treatment methods in patients with advanced head and neck malignancy who had carotid artery involvement and discuss the advantages and disadvantages, immediate or delayed neurologic sequelae, and survival rates. In our report, the operative techniques are discussed and compared with other studies in terms of morbidity and mortality rates and oncological effectiveness.

This study reflects 10 years of experience at the Istanbul University Faculty of Medicine Department of Otorhinolaryngology. In our clinic, patients with head and neck malignancy invading the carotid system were evaluated under elective and emergency conditions. After elective resections, revascularization was performed in selected cases. We observed that in the first 5 years of the period, revascularization procedures were not used frequently, and pharyngostomes were left for protection, whereas revascularization procedures and myocutaneous flaps were widely used in the last 4 years of the period.

MATERIALS AND METHODS

Patients who underwent carotid artery resection for advanced head and neck malignancy between the years 1989 and 1998 were analyzed. Patients who underwent carotid artery resection for other causes were excluded from the study. The total number of patients in the study was 28. The patients with radical extirpation of the invaded carotid artery were divided into 3 groups. Group 1 included patients who underwent immediate resection and thereafter ligation because of rupture of the invaded carotid artery. Group 2 consisted of patients for whom resection without revascularization was performed after preoperative progressive occlusion of the carotid system. In group 3, revascularization was performed after resection.

Carotid artery invasion was actually confirmed surgically and histologically in the elective cases in which angiographic evaluation was performed before the carotid resection (16 patients of group 2 and group 3). If the carotid adventitia that was peeled off during surgery showed any histologic evidence of malignancy, this was accepted as an absolute indication for carotid resection, no matter whether any arterial wall invasion existed.

The sufficiency of the carotid artery system was evaluated in group 2 and group 3 patients (total, 16 patients) with selective angiography during which the backflow of the contralateral side was analyzed by balloon occlusion of the ipsilateral carotid artery. Resection and ligation was the preferred treatment method if preoperative selective angiography demon-

strated adequate perfusion from the contralateral side and if no ischemic neurologic signs developed after balloon occlusion of the invaded carotid artery. However, none of the elective patients (group 2 and group 3) underwent operation according to the decision determined before operation. The surgeon's evaluation of the carotid artery during operation and the backflow stump pressure measurements determined the decision whether to revascularize or to ligate. Resection was considered relatively safe only if the operative backflow stump pressure of the involved carotid artery was sufficient. The carotid stump pressure was measured with a single-channel electrocardiogram monitor with a transducer. A backflow pressure of 70 mm Hg or more was considered safe for resection. A backflow pressure of 50 mm Hg or less was considered to be dangerous for resection, and a pressure between 50 and 70 mm Hg was defined as borderline.

Anastomoses were performed between the common carotid artery and the internal carotid artery in all patients. Patients for whom carotid artery resection was planned received heparin sodium (5,000 U) 2 hours before the operation and 3 times daily for 10 days after the operation. The efficacy of heparinization was evaluated by coagulation time and partial thromboplastin time.

The preoperative variables were patient age, sex, primary tumor, preoperative radiotherapy, and preoperative evaluation. Among the operative variables were the stump pressures of the involved carotid system and the operation and revascularization techniques used. The postoperative variables considered were the morbidity and survival rates. The neurologic outcomes of the patients were evaluated according to the Glasgow Outcome Scale¹² (GOS), which rates the neurologic status of the patient on a scale from 1 to 5 (1, dead; 2, severely disabled; 3, moderately disabled; 4, good recovery; and 5, complete recovery).

The statistical calculations were done by the SPSS (Statistical Package for the Social Sciences) program. The survival rates of the different groups were compared by the Kaplan-Meier life table method. The significance of differences in neurologic morbidity between the group in which revascularization was performed and the group in which resection was performed without revascularization was evaluated with a χ^2 test.

RESULTS

The study consisted of 28 patients with advanced head and neck malignancy who underwent carotid resection between 1989 and 1998. The mean age was 48 years, and the range was 24 to 72 years. The male-

TABLE 2. PATIENTS WITH CAROTID ARTERY INVASION BY ADVANCED HEAD AND NECK MALIGNANCIES FOR WHOM CAROTID RESECTION WAS PERFORMED

Pt. No.	Surgery	Neurologic Deficit Grade	Outcome	Survival (mo)	Cause of Death
1	Saphenous revascularization	None	Unknown	LFU	
2	Immediate resection	None	Died 6 mo	6	Regional recurrence
3	Immediate resection	None	Died 12 mo	12	Regional recurrence
4	Saphenous revascularization	None	Died 1 wk	None	Anastomosis breakdown
5	Gore-Tex revascularization	None	Alive	8	
6	Saphenous revascularization	5	Died 5 mo	5	Local recurrence
7	Gradual ligation and resection	None	Died 12 mo	12	Regional recurrence
8	Saphenous revascularization	None	Alive	12	
9	Gore-Tex revascularization	3	Reached 5-y survival	65	
10	Gradual ligation and resection	None	Died 12 mo	12	Regional recurrence
11	Gradual ligation and resection	5	Reached 5-y survival	65	
12	Immediate resection	4	Died 13 mo	13	Local recurrence
13	Gradual ligation and resection	None	Reached 5-y survival	60	
14	Immediate resection	4	Died 12 mo	12	Regional recurrence
15	Saphenous revascularization	None	Alive	4	
16	Immediate resection	3	Unknown	LFU	
17	Gradual ligation and resection	None	Died 1 mo	1	Local recurrence
18	Immediate resection	None	Alive at 6 mo	LFU 6	
19	Immediate resection	None	Died 12 mo	12	Local recurrence
20	Immediate resection	1	Died 2 d	None	GOS 1 hemiplegia
21	Immediate resection	None	Alive at 24 mo	LFU 24	
22	Gradual ligation and resection	None	Died 18 mo	18	Brain metastasis
23	Gradual ligation and resection	None	Died 6 mo	6	Brain metastasis
24	Immediate resection	None	Died 15 mo	15	Regional recurrence
25	Immediate resection	None	Died 8 mo	8	Regional recurrence
26	Gradual ligation and resection	4	Died 5 mo	5	Local recurrence
27	Immediate resection	None	Died 10 mo	10	Local recurrence
28	Gore-Tex revascularization	None	Died 14 mo	14	GOS 1 hemiplegia

LFU — lost to follow-up; GOS — Glasgow Outcome Scale.¹²

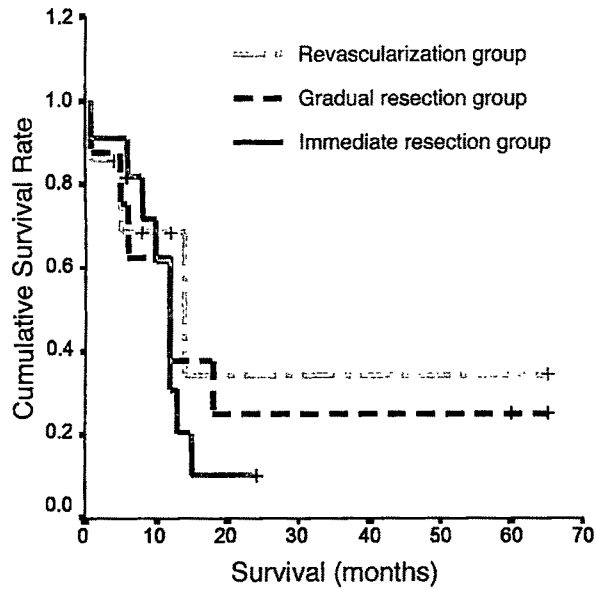
to-female ratio was 2.4 to 1. The locations of the primary tumors were the larynx, hypopharynx, oral cavity, and thyroid gland. Nineteen patients had a history of preoperative radiotherapy. Only 6 of the 28 patients were primary surgical candidates; for the remaining 22 patients, carotid artery resection was performed as a salvage procedure.

Twelve patients required immediate resection because of spontaneous rupture or rupture of the carotid artery during dissection of the tumor off the lamina adventitia of the carotid artery (group 1). In 8 patients, carotid artery resections and ligations were performed after preoperative selective angiograms and the required pressure measurements were made along with progressive occlusion of the carotid system (group 2). Also, in 8 patients (group 3), carotid artery resection and revascularization was performed after preoperative selective angiograms were obtained and inadequate carotid backflow stump pressures were demonstrated during the operation. For

revascularization material, autogenous saphenous vein was used in 5 patients, and the synthetic material Gore-Tex was used in 3 patients. Table 2¹² summarizes the details of the patient information in the series.

In 5 of the 8 patients who underwent revascularization, myocutaneous flap coverage was performed, and in 11 of the 28 patients who underwent carotid resection, a pharyngostoma was left for protection concerns.

Three of the 12 patients who underwent emergency carotid resection were lost to follow-up; 1 of them was free of disease at 6 months and the other at 24 months when they were excluded from the survival evaluation. One of the remaining 9 patients died of hemiplegia (GOS 1) on the second postoperative day, and the others had a mean survival duration of 11 months. These patients died of both local and regional recurrence between 6 and 15 months, and none lived beyond 2 years. Besides the GOS 1 patient who died



Comparison of survival rates between different surgical groups. No statistically significant difference is shown by Kaplan-Meier life table ($p > .05$). Log-rank statistic = 0.73; 2 degrees of freedom; $p = .6946$.

on the second postoperative day, 2 patients experienced GOS 4 hemiplegia and 1 patient had GOS 3 hemiplegia in the postoperative period.

In the second group of 8 patients for whom resection was performed without revascularization after preoperative progressive occlusion of the carotid system, 2 patients were free of disease at 5 years, and the remaining 6 patients died. The overall duration of survival was 22.3 months on average. Two of these patients died of brain metastasis, 2 patients of regional recurrence, 1 patient of local recurrence, and 1 of local residual tumor. Even though all 8 patients in this group were reported as having adequate cerebral perfusion on the balloon occlusion test, and none experienced dangerous intraoperative backflow stump pressures below 50 mm Hg, 1 patient developed GOS 4 hemiplegia and 1 had GOS 5 hemiplegia; both were rehabilitated successfully.

Of the 8 patients in the revascularized group, 1 was lost to follow-up and was excluded from the survival evaluation. One patient who underwent revascularization with saphenous vein died in the first postoperative week because of anastomosis breakdown. The remaining 6 patients had an average duration of survival of 11 months; GOS 5 hemiplegia was observed in 1 patient, and GOS 3 hemiplegia developed in 1 patient. One patient who underwent grafting with Gore-Tex survived for 5 years. One patient died of regional tumor recurrence, and 1 patient with GOS 1 hemiplegia on the appropriate body side died 12 months after the carotid revascularization procedure.

TABLE 3. COMPARISON BETWEEN REVASCULARIZATION AND RESECTION-ONLY GROUPS IN TERMS OF NEUROLOGIC DEFICIT

Surgery	No. of Patients	Neurologic Deficit	No Neurologic Deficit
Resection	20	6	14
Revascularization	8	2	6
Total	28	8	20

No statistically significant difference ($\chi^2 = 0.791$; $p = .52$).

The survival evaluation of the different surgical groups was performed with the Kaplan-Meier life table method. No statistically significant difference was observed among the 3 groups (see Figure).

The neurologic complication rate was compared with the χ^2 test between the revascularization group and the group in which no revascularization was performed, and no significant difference was observed (Table 3). However, it should be noted that 4 of the 8 cases of neurologic complications appeared in patients who underwent immediate resection because of emergent carotid blowout, and thus, no preoperative tests were performed in these patients to evaluate the competence of the contralateral carotid artery system. Two patients who experienced postoperative neurologic complications (one GOS 3 and the other GOS 5) were reported as having insufficient contralateral perfusion of the ipsilateral cerebral hemisphere, and their backflow stump pressure values were less than 50 mm Hg; for this reason, they underwent revascularization. Six of the 8 patients who underwent revascularization experienced no postoperative neurologic complications. The 2 patients who had neurologic complications belonged to group 2 (ligation and resection group). Although both patients were reported as having adequate perfusion from the contralateral side according to the preoperative balloon occlusion test, the operative backflow measurement was borderline for 1 patient, who experienced GOS 4 hemiplegia, and over 70 mm Hg (ie, safe) for the other, who experienced GOS 5 hemiplegia.

DISCUSSION

The prognosis is grim in patients with advanced head and neck malignancy who have carotid artery involvement. The problem is not only to free the patient from the tumor, but also to save the patient from neurologic complications related to the procedure. The chance of these patients' benefiting from chemotherapy, nononcological surgical procedures, external radiotherapy, brachytherapy, or a combination of them is almost zero.^{3,5} The only chance of survival is the radical extirpation of the carotid system invaded by the tumor. This procedure not only offers a chance for cure, but also provides palliation by decreasing pain and local tumor problems.¹¹

If a radical procedure is planned in such patients, carotid artery resection without revascularization or carotid artery resection with bypass grafting may be performed. These surgical methods offer a chance for survival, but also carry a serious risk of neurologic complications. In order to minimize this risk, a good preoperative evaluation is necessary. Preoperative evaluation may not be performed if the patient presents with emergency bleeding or if vessel disruption occurs during dissection of the tumor off the carotid wall. The first step in preoperative evaluation is to determine whether the carotid system is invaded by the tumor. Clinical examination may reveal clues of carotid invasion by the tumor, such as fixation of the mass during vertical movements, but these signs may not always be valid. A mass that was evaluated as being fixed may be resected easily during operation, while a clinically mobile mass may turn out, in the operating room, to have infiltrated the carotid system.⁸ None of the preoperative imaging methods such as ultrasonography, computed tomography, magnetic resonance imaging, or angiography can prove arterial wall invasion by the tumor.^{5,6} The only factor that determines the necessity of radical resection of the tumor is the perioperative observation of the surgeon.⁵

Many techniques have been developed to determine cerebral blood flow sufficiency before operation in order to protect the patient from severe postoperative neurologic complications. The Mats test, duplex ultrasonography, ocular pneumoplethysmography, angiography, the transluminal balloon occlusion test, the balloon occlusion test with cerebral blood flow mapping, the balloon occlusion test with xenon computed tomography, single photon emission computed tomography with technetium-hexamethylpropylene amine oxime, positron emission tomographic scanning with balloon occlusion, and transcranial ultrasonography are such methods.^{5,7,11,13} None of these preoperative methods provides 100% protection from neurologic complications. Use of carotid artery backflow and measurement of carotid stump pressure in the perioperative period also do not offer absolute protection from neurologic complications.^{8,13} This fact is evident in our group 2 patients: 2 experienced postoperative hemiplegias, even though all 8 patients in this group were reported as having adequate cerebral perfusion according to the balloon occlusion test, and none had dangerous intraoperative backflow stump pressure measurements (below 50 mm Hg). Nor does the revascularization procedure offer 100% protection against postoperative neurologic complications. In our series, 6 of the 8 patients in the revascularization group were reported as having insufficient contralateral perfusion

of the ipsilateral cerebral hemispheres and intraoperative backflow stump pressure measurements that were not in the safe zone (over 70 mm Hg). For these reasons, they underwent revascularization. Despite revascularization, 2 patients experienced postoperative neurologic complications. However, it is not clear whether these 2 postoperative neurologic complications were caused by ischemic or embolic insults.

Management of this disease, which carries a high complication rate and has a very complex preoperative evaluation, is very complicated. Although local disease control may be accomplished, long-term survival is not expected. Thus, many researchers in this field have different approaches. Biller et al² have performed resection and bypass grafting with a 7% postoperative neurologic complication rate and a 25% 12-month survival rate. Brennan and Jafek⁵ have performed resection without revascularization with a 29% neurologic complication rate and a mean duration of survival of 7 months. Although, in some studies, the 1-year survival rate has been reported to be as high as 44%, none of the current literature states 3- or 5-year survival rates.⁴ Because morbidity and mortality rates regarding ligation and revascularization vary greatly, there is no consensus among investigators on which method should be used. In our clinic, we have analyzed all available methods that are performed in the setting of carotid invasion. Although the patients were grouped according to preoperative evaluation, and these groups were by no means equal in their ability to pass the preoperative evaluation, we observed that there was no statistically significant difference between the different methods regarding survival rate.

Some investigators do not think resection in these patients is worthwhile; they state that these patients should be considered as having N3 necks and that they have a high risk of distant metastasis.^{5,9} In our series, only 2 of our patients developed distant metastasis. Of the 24 patients with follow-up, 3 have completed 5 years, 1 has completed 2 years, and 1 has completed 1 year of follow-up without signs of disease. If nononcological palliative treatment methods had been chosen for these patients, none of them would have lived that long. In other words, almost 1 of 5 patients with zero chance of survival gained the chance to live by the radical procedure performed. The nonsurvivors benefited from the procedure in terms of palliation. Although it is an undeniable fact that serious complications and death may ensue from aggressive radical surgery, it is the only chance for survival for these patients.

After elective carotid artery resection, the question is whether bypass grafting should be performed.

Some clinicians prefer ligation only because they believe that by this method a more complete tumor resection can be achieved, postoperative radiotherapy can be initiated earlier, and the risk of carotid artery rupture is lower.^{2,5,9} We believe that there is always the risk of embolization of an atheromatous plaque in elderly, atherosclerotic patients in whom vascular operations are performed, and only ligation can offer protection against this risk. Researchers who prefer reconstruction claim that preoperative evaluation by itself cannot eliminate the risk of an intraoperative stroke, so ipsilateral cerebral flow must be preserved.¹⁴ Also, the possibility of future carotid artery disease on the contralateral side should be kept in mind. In our study, we observed that there was no significant difference in the survival rate ($p = .52$) or the neurologic morbidity rate ($\chi^2 = 0.79$) between the ligation and revascularization groups.

CONCLUSION

Internal carotid artery invasion in advanced head

and neck cancers is an unfavorable prognostic sign. Although there are significant postoperative neurologic complications and possible perioperative complications related to the procedure, the only chance of cure for these patients is aggressive resection. The subject of discussion should not be whether resection should be performed, but instead whether revascularization should be performed after resection. Because vascular surgical procedures of the carotid artery carry the risk of many serious complications, including even death, ligation without bypass grafting may be reasonable for patients with adequate contralateral cerebral circulation, and bypass grafting should be reserved for all other elective carotid resection patients. However, in making such a critical decision, both the patient's quality and quantity of life and the opinion of the patient and relatives should be taken into consideration. No statistically significant result has been achieved to prove the superiority of any surgical method carried out after resection.

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CONGENITAL IMPLANTATION CHOLESTEATOMAS OF THE OCCIPITOPARIETOTEMPORAL JUNCTION

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Two cases of cranial cholesteatomas centered at the occipitoparietotemporal junction are presented, and 12 similar cases reported as diploic cholesteatomas involving the temporal bone are reviewed. Among the reported diploic cholesteatomas, 3 involved the occipitoparietotemporal junction and had the same clinical characteristics as the ones presented here. These 5 lesions did not produce expansion of the cranial tables, they had a predominantly intracranial growth, and their matrix was bonded to the dura, so that their complete excision was prevented. It is proposed that cranial cholesteatomas involving the occipitoparietotemporal junction arise, not from within the tables of the skull, but from squamous cells trapped during closure of the mastoid fontanelle and formation of the suture. Hence, they should be recognized as having an origin different from that of diploic lesions, and termed congenital implantation cholesteatomas.

KEY WORDS — cholesteatoma, occipitoparietotemporal junction.

Epidermoid tumors of the skull bones are rare congenital lesions generally believed to develop within the cranial tables. Consequently, they are referred to as diploic or intradiploic cholesteatomas (DCs), a term coined by Muller,¹ who first described them in 1838. Bailey² authored the first report of a DC in the American literature in 1920, and Mahoney³ documented their incidence in 1936. Mahoney found 20 epidermoids (0.65%) in a review of 3,250 intracranial tumors and went on to review the literature on these lesions, noting that only 23 DCs had been described. Since then, DCs have been periodically discussed in the literature, which was updated by Miller et al⁴ in 1994, along with the description of a case of their own. An additional DC has since been reported, for a current total of 75 cases.⁵ The review of Miller et al showed that DCs were relatively more frequent in the frontal (19 or 26%), parietal (15, or 21%), and occipital regions (15, or 21%), while the temporal bone had been involved in only 10 (12%) of the reported cases.

We recently treated 2 patients with cranial cholesteatomas involving the temporal bone. These lesions had some of the characteristics of previously reported DCs, but their clinical and imaging features raised the possibility that they originated not within the tables of the skull, but at the asterion, or occipitoparietotemporal suture. This observation prompted a

review of previously reported cases, which indicated that several DCs involving the temporal bone appeared to originate at the asterion. In this communication, we report the clinical and radiographic findings of our cases, comparing them with those previously reported, and propose an alternative source for their origin.

CASE REPORTS

Case 1. A 63-year-old man presented with a 20-year history of a slowly growing right postauricular mass. The lesion extended anteriorly to within 3 cm of the postauricular crease and posteriorly to within 5 cm of the midline. It was painless, firm, and rounded and had a diameter of 7.5 cm. The patient denied a history of ear disease or trauma. The findings on otomicroscopy were normal on both sides. An audiogram revealed a bilateral, symmetric, high-frequency sensorineural hearing loss with near-normal speech discrimination. Other than hypertension and a recent transurethral prostatectomy for benign disease, the patient was healthy. In the axial projection, a computed tomography scan demonstrated a large, expansile lesion involving the right mastoid process and posterior cranial fossa (Fig 1). Coronal views showed that the lesion also extended into the middle cranial fossa. The attic, middle ear space and its contents, inner ear structures, and facial canal were intact (Fig

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Fig 1. (Case 1) Noncontrast computed tomography scan of temporal bone (axial projection). Note extensive destruction of occipital bone. Arrow indicates mastoid involvement. **Inset)** Coronal section through attic and middle ear documents their normal appearance.

1, inset). The mastoid tip and its air cells were preserved. Magnetic resonance imaging demonstrated a 6 × 7-cm extradural mass involving the medial and posterior cranial fossae. The sigmoid and transverse sinuses were obliterated by the lesion, which was bright on T2-weighted sequences and gave an intermediate signal on T1-weighted images (Figs 2 and 3).

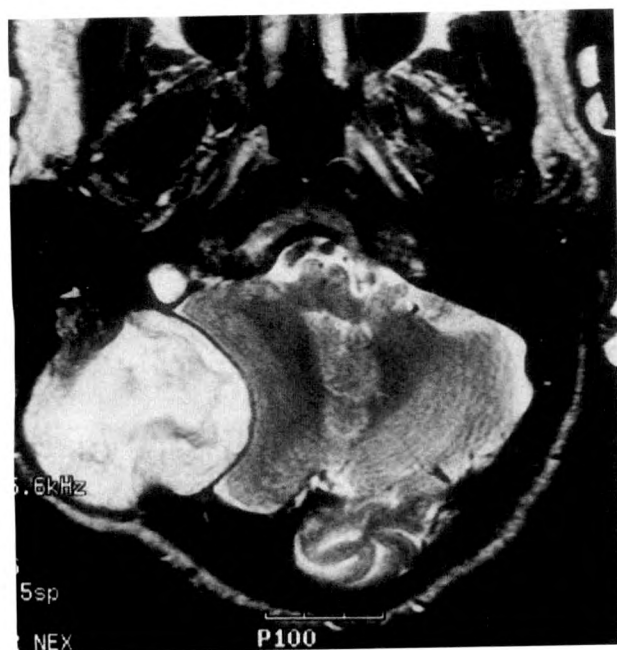


Fig 2. (Case 1) T2-weighted magnetic resonance image in axial projection demonstrates extradural nature of lesion. Bright appearance of mass is characteristic of cholesteatoma.

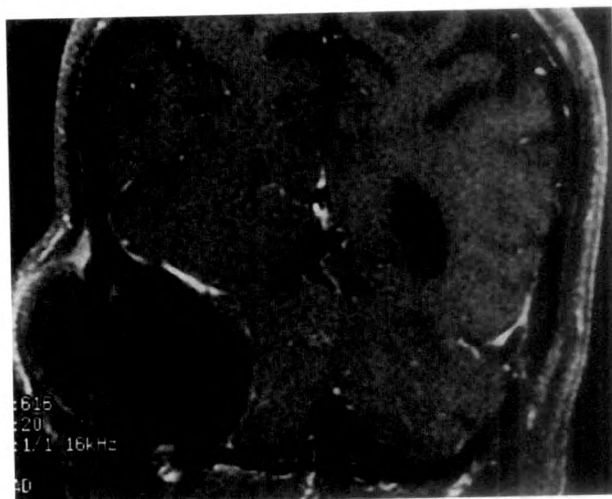


Fig 3. (Case 1) T1-weighted magnetic resonance image in coronal projection further demonstrates extradural nature and massive size of lesion.

The mass was explored through a large C-shaped midoccipital incision. Its extradural nature was confirmed, and its limits were delineated. Since the matrix of the mass in contact with the dura was bonded to it, complete excision would have required resection of the posterior and middle fossa dura, along with the transverse and sigmoid sinuses and the vein of Labbe. Therefore, it was elected to evacuate the lesion's keratinaceous contents and to resect its lateral lining to its point of contact with the dura and mastoid cavity. In the temporal bone, the lesion extended to the antrum, but did not involve the aditus, and there was no disease extending below the dome of the lateral semicircular canal. The attic and its contents were normal. At the extreme anterior extension of the lesion (antrum), no matrix was present, but a small amount of free squamous debris was encountered. The posterior superior external ear canal wall was left intact. The air cells posterior to the attic were exenterated along with those of the mastoid tip and retrofacial space. Thereafter, a large temporalis fascia graft was harvested and placed so as to separate the lateral extent of the attic from the evacuated mass. The bony defect was repaired with a lightweight titanium mesh. Histologic study confirmed the diagnosis of cholesteatoma. The patient has done well and had no new sensory or neurologic deficits for 2 years after surgery. Imaging performed 14 months after surgery showed a small amount of residual cholesteatoma at the surgical site. Lifelong follow-up is planned.

Case 2. A 35-year-old man presented with a 12-month history of headaches. He was otherwise in good health and had no other symptoms. A physical examination was significant for a 2 × 2-cm, slightly tender defect located 4 cm posterior to the postauricular sulcus in the region of the right occipitoparietotemporal suture. Magnetic resonance imaging docu-

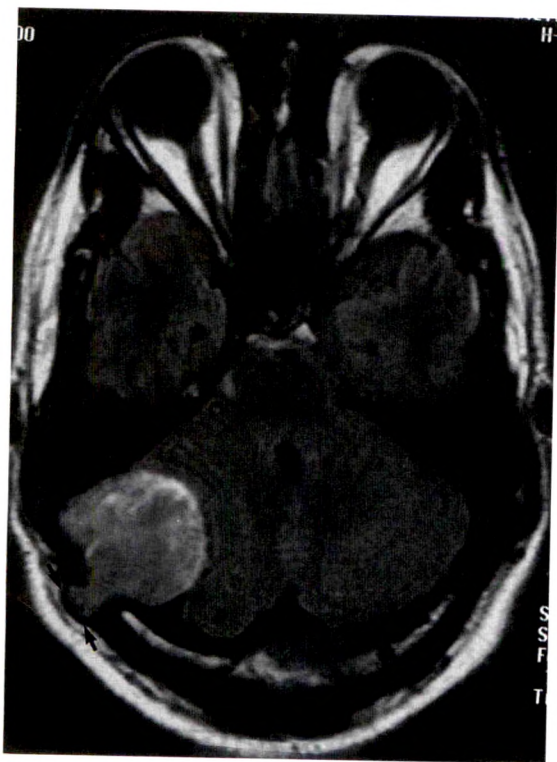


Fig 4. (Case 2) T2-weighted magnetic resonance image in axial projection. Note lack of extracranial expansion of lesion. Arrows point to skull defect documented at surgery as corresponding to asterion.

mented this defect and showed a 3.5×3.5 -cm lesion in the posterior cranial fossa, abutting the posterior edge of the mastoid process and extending superiorly to the level of the tentorium (Fig 4). The lesion was operated upon through a postauricular incision placed just behind the skull defect, which was circumferentially enlarged to expose the whole extent of the lesion. The retrosigmoid air cells were entered, but they were not involved by the mass. It contained dense, highly packed squamous debris and was histologically confirmed as a cholesteatoma (Fig 5). The lesion was completely evacuated, but for reasons similar to those in case 1, block resection of its matrix was limited to the portion without dural contact. Under high magnification, fragments of the matrix that could be peeled off the dura without risk of breaching it were excised in an effort to prolong the recurrence interval. The patient did well after surgery. Yearly imaging studies are planned.

DISCUSSION

The cholesteatomas reported here may be interpreted as the same type of lesion with a difference of 30 years in evolution. The extramastoid origin of case 2 is indisputable, but case 1 could be misdiagnosed as an acquired or congenital mastoid cholesteatoma extending intracranially. Acquired disease is readily excluded, since the patient had a normal middle ear

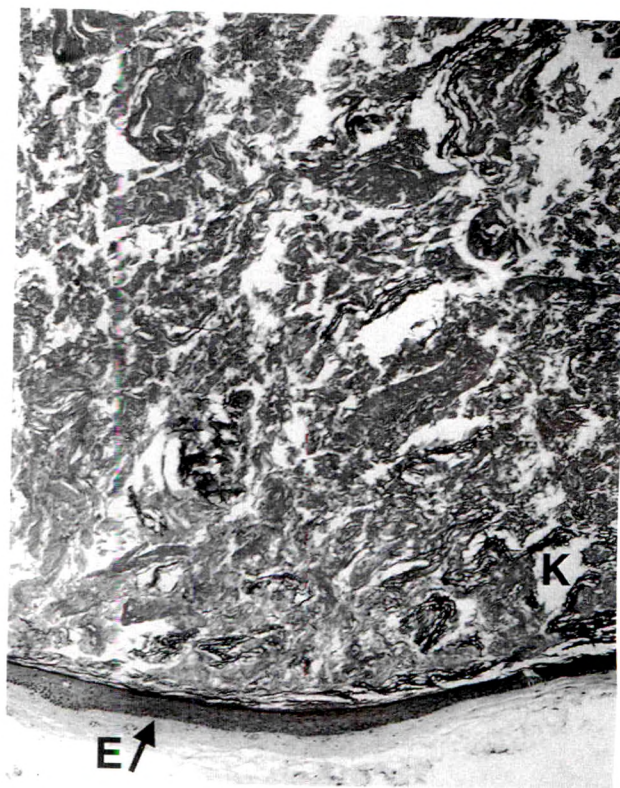


Fig 5. (Case 2) Photomicrograph of pathological findings (H & E, original $\times 10$). Mass is lined by squamous epithelium (E), and its contents are composed of lamellar keratin (K).

and no history of otitis media, but the possibility of a congenital temporal bone lesion merits further scrutiny. A probable source for congenital cholesteatomas was identified by Michaels⁶ in the epidermoid formation, an epithelial middle ear tuft present in 10- to 33-week-old human fetuses. Cholesteatomas deriving from involutional failure of this remnant would arise in the anterior mesotympanum and grow to involve the eustachian tube and ossicles and, in advanced stages, the mastoid air cells. Michaels⁷ also proposed that caudal migration of the epidermoid formation could occur and result in more posteriorly located lesions, but recent work does not support this hypothesis. Cytokeratin analysis of 20 human temporal bones ranging in age from 20 weeks' gestation to 8 months' postnatal age by Liang et al⁸ showed that the epidermoid formation is confined to the lateral wall of the anterior half of the middle ear cavity. Other, more posteriorly located epithelial thickenings included in this study failed to show expression of cytokeratins 10 and 14, thereby excluding themselves as possible cholesteatoma sources. It is clear, then, that the mastoid involvement in case 1 was a consequence of the growth of a cranial cholesteatoma with a more caudal point of origin.

To date, there have been 12 cranial lesions with temporal bone involvement reported as DCs^{4,9-18} (see

CRANIAL CHOLESTEATOMAS WITH TEMPORAL BONE INVOLVEMENT

Authors	Year	Age (y)	Sex	Site	Size (cm)	Symptoms	Intracranial Extent	Extracranial Extent
Cushing ⁹	1922	40	M	TPO L	13	Mass	4+	1+
King ¹⁰	1939	28	M	TPF R	7	Hemiparesis, diplopia	4+	1+
Monroe and Wegner ¹¹	1937	37	F	T R	NA	Headaches	NA	NA
Rand and Reeves ¹²	1943	54	F	T R	4.5	Seizures	1+	2+
Tytus and Pennybacker ¹³	1956	60	M	FT R	NA	Seizures	4+	1+
Canale et al ¹⁴	1974	46	M	TPO R	14	Mass, headaches	4+	1+
Nager ¹⁵	1975	21	F	FT L	4	Mass	1+	3+
Constans et al ¹⁶	1985	59	M	TPO R	7	Mass, headaches	3+	3+
Rubin et al ¹⁷	1989	21	F	T R	NA	Headaches	NA	NA
		17	M	TP R	NA	Mass	NA	NA
Ciappetta et al ¹⁸	1990	14	F	T L	NA	Mass	NA	NA
Miller et al ⁴	1994	19	M	T L	2.5	Mass	1+	2+
Present study		63	M	TPO R	7.5	Mass	4+	2+
		35	M	TPO R	3.5	Headaches	4+	—

T — temporal; P — parietal; O — occipital; F — frontal; NA — not available; — — none; 1+ — minimal; 2+ to 3+ — moderate; 4+ — more than 4 cm.

Table). Half^{4,12,16,17} were described as limited to the temporal bone, but an analysis of these cases indicated that at least 1, measuring 7 cm in diameter,¹⁶ also involved the parietal and occipital bones. Of the remaining 6, 2 appeared centered at the asterion,^{9,14} and 4 were anteriorly located and predominantly involved the frontal bone^{10,13,15} or parietal bone.¹⁷ In the 5 DCs limited to the temporal bone and in the 4 with predominantly frontal or parietal involvement, there was expansion of the tables of the skull, and their main growth was extracranial. At surgery, the inner table remnant was usually dehiscent, but provided enough separation from the dura to allow for a complete resection of the cholesteatoma. The 3 lesions involving the temporal, parietal, and occipital bones^{9,14,16} were very similar to the 2 described here. They were the largest among the reported DCs, they had no remnants of either cranial table surrounding them, and their predominant growth was intracranial. At surgery, their matrix was intimately attached to the dura, preventing their complete excision, and their epicenter (especially noticeable in our case 2) appeared to be the occipitoparietotemporal junction, which is the location of the mastoid fontanelle in the newborn.

Given that the clinical features of the 5 cranial cholesteatomas centered at the asterion differed significantly from those generally ascribed to DCs, it is reasonable to consider a different mechanism for their origin. The more logical explanation is that they arose from squamous epithelium trapped within the suture

sometime during the closure of the mastoid fontanelle.

The development of cranial sutures is a complex and prolonged process that begins in early embryogenesis and ends in the third decade of life, when their fusion is completed.¹⁹ Sutures are major sites of intramembranous osteogenesis, but in order to function as such, they need to remain patent, while allowing rapid bone formation at the edges of their bone fronts.^{20,21} During this process, the bone fronts invade and recruit the intervening mesenchyme into their advancing edges, thereby separating it into an outer, periosteal layer and an inner, dural layer.²⁰ Both layers appear related to bone development, but the ectoperiosteum may also be associated with forming dermis.^{21,22}

Fontanelles occur where more than 2 cranial bones meet and are constituted by dura, periosteum, and skin. Their closure likely follows steps similar to those of simple sutures, wherein their persistent patency, along with active involvement of all primitive layers, may provide a setting in which trapping of ectodermally derived squamous cells can occur. Cholesteatomas resulting from such entrapment would have the characteristics of the ones presented here, and should be best defined as implantation or suture cholesteatomas, rather than DCs. Epithelial implantation as a source of congenital cholesteatomas has been previously hypothesized by Friedmann,²³ but to our knowledge, evidence supporting it has not been previously reported.

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COMPLICATION-FREE LARYNGEAL SURGERY AFTER IRRADIATION FAILURE WITH PROSTAGLANDIN E1 ADMINISTRATION

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To examine the usefulness and efficacy of administration of prostaglandin E1 (PGE₁) after laryngeal surgery in patients who were previously treated with radiotherapy, we retrospectively examined the clinical data of 12 patients who had undergone partial laryngectomy and 21 patients who had undergone total laryngectomy. Complications were observed in 5 of the 7 cases treated with partial laryngectomy without PGE₁ administration, while no complications were observed in the 5 cases treated with PGE₁ after operation. Also, complications, including major leakage, were observed in 6 of the 15 patients who underwent total laryngectomy without PGE₁, and no complications were observed in the 6 patients who received PGE₁. The hospital stay was shorter for the patients treated with PGE₁ than for those not receiving such treatment. Although this study was a small, nonrandomized pilot trial, the results indicated that PGE₁ administration may be useful and effective for patients who undergo laryngeal surgery after irradiation failure, in decreasing the risk of postoperative complications and increasing the quality of life of the patients.

KEY WORDS — complication, irradiation failure, laryngeal cancer, prostaglandin E1.

INTRODUCTION

Radiotherapy is the primary treatment of choice for early glottic cancer of the larynx in many hospitals, because it has a high rate of efficacy associated with a low rate of morbidity and retention of a reasonably normal voice.¹⁻⁵ In a small number of patients in whom radiotherapy has failed to control the tumor, surgery represents a salvage option.⁶⁻¹³ Laryngeal surgery (corpectomy, partial laryngectomy, or total laryngectomy) is needed for patients who fail to respond to radiotherapy. However, many studies have reported an increased incidence of postoperative complications such as necrosis of the skin flap, leakage, cutaneous fistula, wound infection, abscess formation, and perichondritis in such patients as compared with those treated initially with surgery.

Prostaglandin E1 (PGE₁) is a prostanoid known to relax the blood vessels and increase the blood flow.^{14,15} Although its potential for improving preoperative, perioperative, and postoperative status has been reported in animal models, as well as in patients after various types of surgery, there have been few reports describing its results in patients who have undergone laryngeal surgery after irradiation failure.

Herein, we report the usefulness and effectiveness of PGE₁ administration after laryngeal surgery in patients who had been previously treated with radiotherapy.

PATIENTS AND METHODS

Twelve patients with laryngeal cancer who failed

to respond to radiotherapy underwent partial laryngectomy at Miyagi Cancer Center Hospital from November 1993 to April 2000. The surgical procedure, vertical frontolateral partial laryngectomy, was performed according to the method described by Hayashi et al¹³ and by Denecke.¹⁶ Meanwhile, total laryngectomy was conducted in 21 patients with laryngeal cancer who failed to respond to radiotherapy in our hospital from July 1993 to January 2001. The same surgeons performed all of the operations during these periods. On the basis of histologic examination, all of the removed tumors were diagnosed as squamous cell carcinoma. Prostaglandin E1 at 120 µg/d was administered intravenously in 5 of the 12 patients after partial laryngectomy starting in September 1998, and in 2 of the 21 patients after total laryngectomy starting in April 1999, for a maximum duration of 2 weeks. The same dosage of PGE₁ was administered intravenously to 4 patients who underwent total laryngectomy, before and after operation, to reduce the duration of PGE₁ administration, starting in June 2000. Detailed profiles of the patients are presented in Tables 1 and 2. The only factor used to select patients for PGE₁ treatment was the period in which they underwent surgery. There was no significant difference between the patients who received PGE₁ and those who did not as to age, physical status, total dose of radiation, or interval between cessation of radiotherapy and surgical treatment. The patients listed in Table 2 as cases 18 and 21 are the same patients listed in Table 1 as cases 8 and 10, respectively.

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TABLE 1. PATIENTS WHO UNDERWENT PARTIAL LARYNGECTOMY AFTER RADIOTHERAPY

Patient	Age (y)	Sex	TNM Classification*	Date of Operation	Total Dose of Radiation and Interval Before Operation	Length of PGE ₁ Administration (d)	Postoperative Complications	Hospital Stay After Surgery (d)
1	75	M	T2N0M0	Nov 17, 1993	80 Gy, 2 y (other hospital)	None	Flap necrosis, infection	89
2	58	M	T1aN0M0	Apr 14, 1995	62 Gy, 8 mo	None	Ulcer formation	29
3	39	M	T1aN0M0	Feb 2, 1996	67.5 Gy, 2 mo	None	None	25
4	69	M	T1aN0M0	May 30, 1997	70 Gy, 4 mo (other hospital)	None	Perichondritis	34
5	59	M	T2N0M0	Jun 11, 1997	70 Gy, 2 mo	None	Flap necrosis, infection	18
6	69	M	T2N0M0	Feb 27, 1998	70 Gy, 11 mo	None	Flap necrosis, infection	18
7	66	M	T1aN0M0	Jul 31, 1998	68 Gy, 2 y	None	None	10
8	47	M	T1bN0M0	Sep 11, 1998	80 Gy, 5 mo (other hospital)	13	None	19
9	70	M	T3N0M0	Oct 16, 1998	70 Gy (+ chemotherapy), 9 mo	14	None	17
10	51	M	T1aN0M0	Jul 23, 1999	65 Gy, 3 y 5 mo	14	None	15
11	80	M	T1aN0M0	Feb 2, 2000	70 Gy, 3 mo (other hospital)	6	None	9
12	63	M	T2N0M0	Apr 11, 2000	60 Gy, 3 y 1 mo (other hospital)	8	None	11

PGE₁ — prostaglandin E₁.

*TNM classification was made before initial treatment (radiotherapy).

Statistical analyses of the data were performed by χ^2 test and *t*-test.

RESULTS

Of the 7 patients who underwent partial laryngectomy without PGE₁ administration after surgery, 5 showed postoperative complications: necrosis of the skin graft and infection in 3 patients, ulcer formation in 1 patient, and perichondritis in 1 patient (Table 1). The total dose of radiation in these patients ranged from 62 to 80 Gy, and the time from initial treatment to the salvage operation ranged from 2 months to 2 years. The hospital stay after partial laryngectomy was 10 to 89 days, with a mean of 31.9 days.

Of the 5 patients who underwent partial laryngectomy and had postoperative PGE₁ administration, none showed postoperative complications. The absence of side effects or complications is considered to be due to the PGE₁ administration. The skin of all patients who received PGE₁ was flushed from dilatation of the skin capillaries — a finding suggesting that PGE₁ increased the blood supply to their skin (Figs 1 and 2). There was no evidence of cellulitis, abscess, or chondritis, and the patients experienced no pain, fever, leukocytosis, or purulent discharge from the wound during the postoperative period. The total dose of radiation in these patients was between 60 and 80 Gy, and the time interval from initial treat-

ment to the salvage operation ranged from 5 months to 3 years 10 months. The hospital stay after partial laryngectomy was 9 to 19 days, with a mean of 14.2 days.

The incidence of postoperative complications was significantly higher in the patients without PGE₁ administration than in the patients with PGE₁ administration ($p = .013$). Although statistical analysis showed no significant difference, the postoperative hospital stay of the patients who received PGE₁ was shorter than that of the patients who did not (Table 3). The PGE₁ administration caused no side effects such as hemorrhagic tendency or hypotension.

Of the 15 patients who underwent total laryngectomy after irradiation failure without PGE₁ administration, 6 showed postoperative complications: major leakage followed by cutaneous fistulas in 5 patients and subcutaneous necrosis and infection in 1 patient. The total dose of radiation in these patients was between 40 and 80 Gy, and the interval from the initial treatment to the salvage operation ranged from 1 month to 9 years 8 months. The hospital stay after total laryngectomy was 17 to 77 days, with a mean of 33.8 days.

Of the 6 patients who underwent total laryngectomy with (preoperative and) postoperative PGE₁ administration, none had postoperative complications.

TABLE 2. PATIENTS WHO UNDERWENT TOTAL LARYNGECTOMY AFTER RADIOTHERAPY

Patient	Age (y)	Sex	TNM Classification	Date of Operation	Total Dose of Radiation and Interval Before Operation	Length of PGE1 Administration (d)	Postoperative Complications	Hospital Stay After Surgery (d)
1	61	M	rT3N0M0	Jul 28, 1993	40 Gy, 1 mo (other hospital)	None	Wound infection, abscess	77
2	75	M	rT3N0M0	Jun 8, 1994	45 Gy, 7 y; 35 Gy, 4 y (other hospital)	None	Leakage, cutaneous fistula	36
3	63	M	rT3N0M0	Sep 28, 1994	64 Gy, 5 mo	None	None	21
4	67	M	rT4N0M0	Feb 24, 1995	? , 8 y 5 mo (other hospital)	None	None	17
5	70	M	rT1bN0M0	Jun 1, 1995	36 Gy, 5 y; 30 Gy, 1 y 4 mo (other hospital)	None	None	21
6	58	M	rT4N0M0	Apr 5, 1996	70 Gy, 3 y 10 mo	None	Leakage, cutaneous fistula	61
7	39	M	rT4N0M0	Oct 23, 1996	67.5 Gy, 11 mo	None	None	22
8	63	M	rT2N0M0	Jan 17, 1997	60 Gy, 3 y	None	None	26
9	65	M	rT3N0M0	Feb 28, 1997	70 Gy, 5 y 7 mo (other hospital)	None	Leakage, cutaneous fistula	30
10	62	M	rT2N0M0	May 7, 1997	66 Gy, 8 mo	None	None	17
11	60	M	rT3N0M0	May 21, 1997	70 Gy, 1 y 8 mo	None	None	21
12	74	M	rT2N0M0	Aug 22, 1997	68 Gy, 1 y 3 mo	None	None	15
13	55	M	rT2N0M0	Dec 10, 1997	70 Gy, 3 mo	None	Leakage, cutaneous fistula	58
14	66	M	rT2N0M0	Aug 19, 1998	71 Gy, 5 y 5 mo	None	None	19
15	68	M	rT2N0M0	Apr 27, 1999	40 Gy, 9 y 8 mo (other hospital)	None	Leakage, cutaneous fistula	66
16	63	M	rT2N0M0	Apr 30, 1999	70 Gy, 2 mo	14	None	17
17	65	M	rT2N0M0	May 19, 1999	70 Gy, 11 mo	14	None	24
18	48	M	rT2N0M0	Jun 2, 2000	80 Gy, 2 y 2 mo (other hospital)	9*	None	18
19	83	M	rT1bN0M0	Sep 2, 2000	65.5 Gy, 4 y 4 mo	10*	None	27†
20	62	M	rT2N0M0	Nov 17, 2000	70 Gy, 2 y (+ chemotherapy)	11*	None	20
21	53	M	rT2N2cM0	Jan 5, 2001	65 Gy, 4 y 10 mo	9*	None	15

*Including preoperative administration.

†This patient had irritable colon after operation.

Again, the absence of complications is thought to be due to PGE1 administration. The total dose of radiation in these patients was between 65 and 80 Gy, and the interval from the initial treatment to the salvage operation ranged from 2 months to 4 years 10 months. The hospital stay after total laryngectomy was 15 to 27 days, with a mean of 20.2 days.

Incidentally, patients who underwent total laryngectomy without PGE1 administration tended to have a higher T stage (T1-T4) than did those who received PGE1 (T1-T2). The incidence of postoperative complications was higher in the patients without PGE1 administration than in those with PGE1 administration, although no significant difference was observed by statistical analysis. Although statistical analysis showed no significant difference, the postoperative hospital stay of the patients who received PGE1 was shorter than that of the patients who did not (Table 3).

Finally, the incidences of postoperative complications and the lengths of the postoperative hospital stay after all laryngeal surgeries were compared between the patients, ie, those treated with partial and total laryngectomy with and without PGE1 administration. The incidence of postoperative complications was significantly higher in the patients without PGE1 administration than in those with such administration ($p = .0041$). Although statistical analysis showed no significant difference, the postoperative hospital stay of the patients who received PGE1 was shorter than that of the patients who did not ($p = .058$; Table 3).

DISCUSSION

This is the first report describing the usefulness and efficacy of PGE1 administration after laryngeal surgery in patients who had not responded to radiotherapy. It should first be mentioned that the hospital stay of patients after surgery tends to be longer in

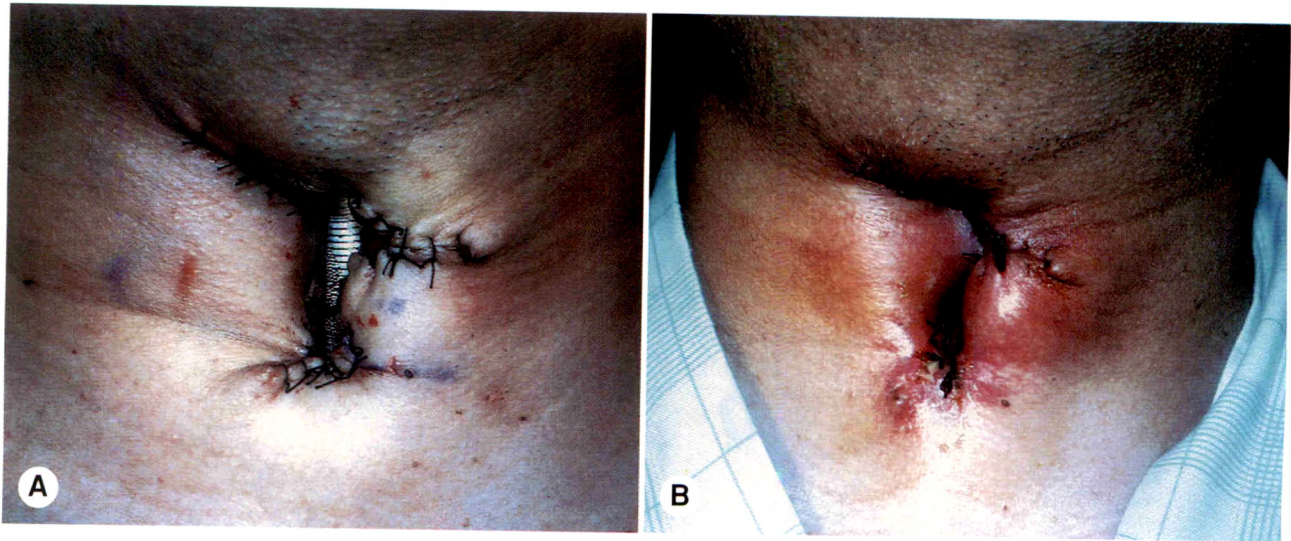


Fig 1. Fifty-one-year-old man (patient 10 in Table 1) who underwent partial laryngectomy at our hospital on July 23, 1999. **A)** Local findings just after surgery. Laryngostomy was performed, and endotracheal tube can be seen. **B)** Local findings on 10th postoperative day. Flushing was observed in skin around wound. There were no complications such as necrosis of skin flap, infection or abscess, or perichondritis.

Japan than that of patients in other areas such as Europe, because of differences in the insurance systems.

Many patients who fail to respond to radiotherapy against laryngeal cancer suffer from postoperative complications such as leakage, fistula formation, skin necrosis, perichondritis, and abscess formation. In our hospital, we have so far experienced no postoperative wound complications in patients with laryngeal cancer who were initially treated with laryngeal surgery. Furthermore, there have been previous reports of various postoperative complications of laryngeal surgery after irradiation failures.⁶⁻¹³ For example, Hayashi et al¹³ reported a high frequency (42%)

of postoperative complications after partial laryngectomy. As they used almost the same method that we used for our patients, reference to their results was useful in assessment of our results. The rate of complications for the patients who did not receive PGE1 was extremely high in this study. Perhaps this was a result of the operative procedure we use, in which the tension of the skin flap is very high because of the formation of a laryngostoma. These reported complications were thought to be brought about by insufficiency of microcirculation in the skin or mucosa due to the patients' prior radiotherapy.

The effects of PGE1 on the blood flow of arteries, arterioles, and capillaries have been well studied in

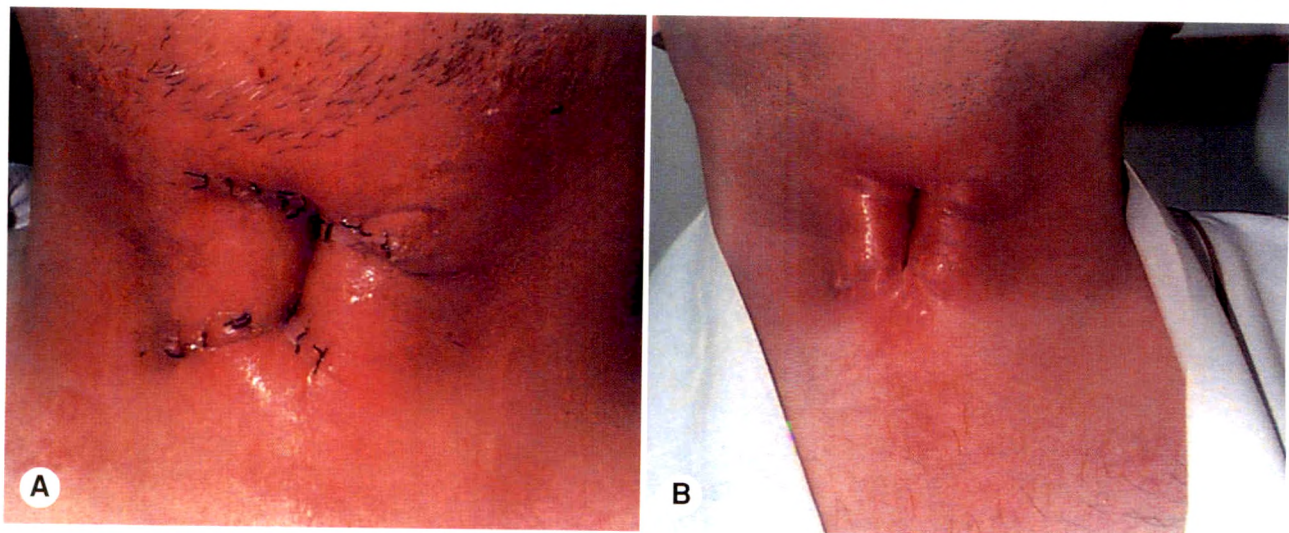


Fig 2. Forty-seven-year-old man (patient 8 in Table 1) who underwent partial laryngectomy at our hospital on September 11, 1998. **A)** Local findings on 6th postoperative day. Laryngostomy was performed. **B)** Local findings on 18th postoperative day. Flushing was observed in skin around wound. There were no complications such as necrosis of skin flap, infection or abscess, or perichondritis.

TABLE 3. COMPLICATIONS AND HOSPITAL STAY AFTER SURGERY

	No. of Patients	Complications		Hospital Stay After Surgery (d; mean $\pm$ SD)
		+	-	
Partial laryngectomy after radiotherapy				
With PGE ₁	5	0 (0%)	5 (100%)	14.2 $\pm$ 3.7
Without PGE ₁	7	5 (71%)	2 (29%)	31.9 $\pm$ 26.4
		p = .013*		p = .102†
Total laryngectomy after radiotherapy				
With PGE ₁	6	0 (0%)	6 (100%)	20.2 $\pm$ 4.1
Without PGE ₁	15	6 (40%)	9 (60%)	33.8 $\pm$ 21.7
		p = .067*		p = .16†
All laryngeal surgery after radiotherapy				
With PGE ₁	11	0 (0%)	11 (100%)	17.5 $\pm$ 4.9
Without PGE ₁	22	11 (50%)	11 (50%)	33.2 $\pm$ 21.6
		p = .0041*		p = .058†

* χ^2 Test.
†t-Test.

* χ^2 Test.

†t-Test.

animal models.^{14,15,17} There have been several studies concerning such effects in humans. Prostaglandin E1 has been found to be useful in alleviating ischemia in patients with peripheral arterial occlusive disease,^{18,19} improving blood flow after esophagectomy,^{20,21} maintaining patients after cardiovascular surgery,^{22,23} performing free flap salvage,²⁴ and so on. However, to date, there have been no studies clarifying the effects of PGE1 in postoperative patients who failed to respond to irradiation. In our case, the blood supply to the skin of the patients dramatically increased, resulting in flushing due to dilatation of the skin capillaries (Figs 1 and 2). This may be the first report to show macroscopically the effect of PGE1 on the skin of patients who undergo laryngeal surgery after irradiation failure.

It should be emphasized that there were no apparent side effects or complications of PGE1 administration in our study. However, potential side effects such as hypotension, bleeding from the wound, pulmonary edema, leukocytopenia, and phlebitis have been clinically noted. Perhaps our fixed dosage of 120 μ g/d is appropriate in such patients for avoiding side effects and complications.

Our finding that postoperative complications can be effectively curtailed by PGE1 administration is good news for patients who have failed to respond to radiotherapy.

Unfortunately, the number of subjects we were able to study was restricted, because this trial was limited to patients in whom surgery was carried out after failure of initial radiotherapy. However, even though the number of patients was small, significant differences regarding postoperative complications were shown by statistical analysis in the patients who underwent partial laryngectomy. No significant difference was observed in the patients who underwent total laryngectomy because of the low number of patients who received PGE1. Moreover, it should be mentioned that as patients who underwent total laryngectomy without PGE1 administration tended to have higher T stages than those who received PGE1 administration, it is possible that there may be other reasons why patients with the more advanced tumors tended to have more wound healing problems. However, it is likely that the PGE1 administration was useful and effective for the avoidance of postoperative complications in the patients who underwent total laryngectomy, as well as in those who underwent partial laryngectomy, as there were no postoperative complications after surgery and the postoperative hospital stay was shorter for the patients who received PGE1. As this study was a small, nonrandomized pilot trial, a larger study with randomization of the patients and a larger number of patients is needed for assessment of the usefulness and effectiveness of PGE1 administration in patients who undergo laryngeal surgery after irradiation failure.

We intravenously administered PGE1 to the patients for 2 weeks at maximum. The medical cost to the patients who underwent partial laryngectomy with postoperative PGE1 administration was lower than that of the patients who did not receive PGE1 (data not shown). However, no significant difference as to medical cost was observed between these two groups. In patients with laryngeal cancer who fail to respond to radiotherapy and subsequently undergo surgery, PGE1 administration at an appropriate dosage should lead to lower medical costs and an improved quality of life.

CONCLUSION

Administration of PGE1 may have effects that lead to decreased postoperative complications in patients who undergo laryngeal surgery after irradiation failure. Further basic and clinical research is required to more fully investigate this possibility.

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FIBEROPTIC LARYNGEAL SURGERY FOR VOCAL PROCESS GRANULOMA

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We developed a technique of fiberoptic laryngeal surgery for the treatment of vocal process granulomas. In this system, the granuloma can be removed relatively easily and repeatedly under topical anesthesia on an outpatient basis. We treated 27 patients for a total of 4 intubation granulomas and 23 contact granulomas. Ten of the 23 contact granulomas recurred after the initial surgery, but the intubation granulomas did not recur. Most of the recurrent lesions were resolved by fewer than 3 procedures, and all patients were finally cured. Although conservative therapies such as voice therapy and proton pump inhibitors have recently prevailed, surgical removal remains useful in treating vocal process granulomas. Fiberoptic laryngeal surgery facilitates repeated surgical procedures.

KEY WORDS — fiberoptic laryngeal surgery, topical anesthesia, vocal process granuloma.

INTRODUCTION

Granulomas of the larynx usually occur on the vocal process, and can be classified in terms of the causative factor: intubation, hyperfunctional, and hyperacidic granulomas.¹ So-called contact granuloma involves hyperfunctional and hyperacidic granulomas. Postintubation granuloma often arises after prolonged intubation. The intubation tube can induce injury to the epithelial mucosa of the vocal process.

Jackson² described contact ulcer on the vocal process that might be derived from vocal abuse and might cause a granuloma. Patients with contact granulomas tend to speak in a low-pitched voice with a hyperfunctional phonatory pattern that should increase the force between the vocal processes when they contact each other during phonation. Hyperacidic granuloma has been recognized since Cherry and Margulies³ postulated the etiologic factor of vocal process granuloma to be gastroesophageal reflux (GER). More recently, it was confirmed that gastric acid easily bathes the posterior glottis in GER patients, and thus may cause a chronic inflammatory change in the posterior glottis.⁴ Contact granuloma can arise more frequently in these patients.

In the treatment of vocal process granuloma, surgical removal was formerly common, but the outcome of surgery was disappointing because of the high rates of recurrence, especially of hyperfunctional and hyperacidic granulomas. The recent prevailing treatment is voice therapy in patients with hyperfunctional granuloma, and antacid therapy for hyper-

acidic granuloma. Surgical removal has come to be avoided, and some authors have even considered surgery to be contraindicated.^{1,5} It is certain that voice therapy and antacid treatments have good effects on some patients. However, resolving granuloma by these conservative therapies usually takes a long time, and the therapeutic results have not always been sufficient.

The reason surgery has been resisted for refractory granuloma may be that endoscopic microsurgery under general anesthesia would be necessary for each recurrence, and would impose considerable economic and physical burden on the patients. Additionally, the intubation tube obstructs the surgical view of the posterior glottis, hence making the procedure more difficult and possibly insufficient. We have developed fiberoptic laryngeal surgery with topical anesthesia for phonomicrosurgical treatment.^{6,7} This procedure enabled us to perform precise laryngeal surgery more easily as outpatient surgery, and thus has helped us carry out surgical treatment repeatedly for a refractory lesion. Furthermore, vocal process granuloma is a good indication for this procedure, because there is no obstruction such as an intubation tube in the surgical field. After 1991, we adopted this surgical method as our main strategy for treating vocal process granuloma, with or without other conservative remedies. In the present report, we review the surgical results of this procedure retrospectively, and discuss the strategies used in treating laryngeal granuloma.

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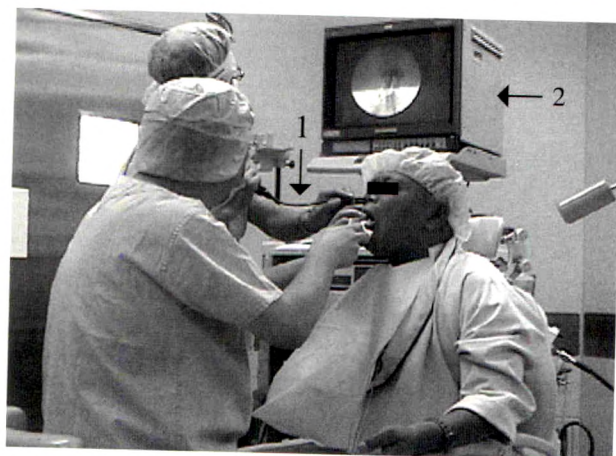


Fig 1. System of fiberoptic laryngeal surgery under topical anesthesia. 1 — fibroscope; 2 — monitor.

MATERIALS AND METHODS

Patients. Twenty-eight patients with vocal process granuloma underwent fiberoptic laryngeal surgery under topical anesthesia in our department between 1991 and 1999. Twenty-one were male, and the other 7 were female. Their ages ranged from 23 to 74 years, with a mean age of 54 years.

Five patients had granulomas following intubation. The granulomas were unilateral in 3 cases and bilateral in the other 2. Two patients had unilateral hyperfunctional granulomas as a result of vocal abuse. Twenty-one patients were considered to have unilateral granulomas caused by reflux laryngitis. Reflux was determined by endoscopic laryngeal findings and reflux symptoms. The endoscopic findings involved thickened interarytenoid mucosa, so-called pachydermia, erythema and edema of the arytenoid cartilages, and infraglottal edema. The symptoms included chronic cough, throat irritation, globus sensation, throat pain, and hoarseness. A patient with at least 1 endoscopic finding accompanied by at least 1 reflux symptom was considered to have acidic granuloma. The group with hyperfunctional granuloma consisted of patients with hypertonic movement of

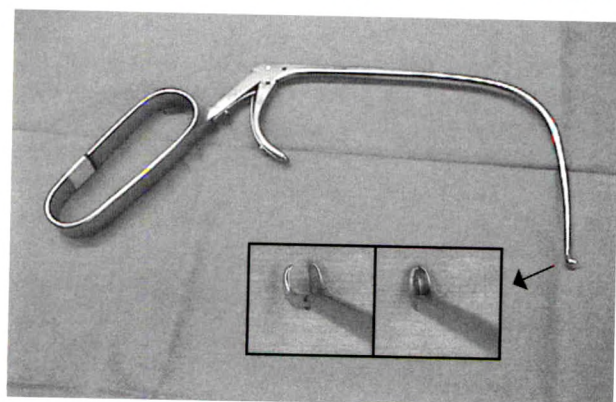


Fig 2. Specially developed fine-tipped forceps. Inset) Tip opens in horizontal plane.

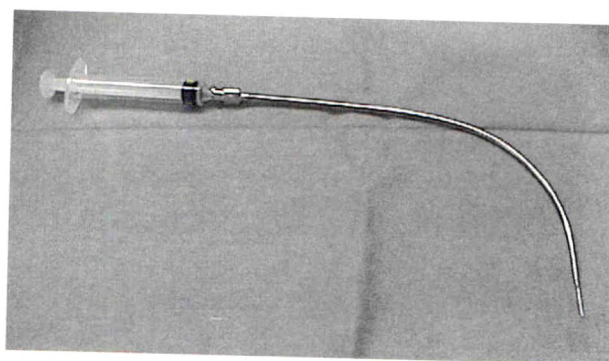


Fig 3. Curved needle for steroid injection.

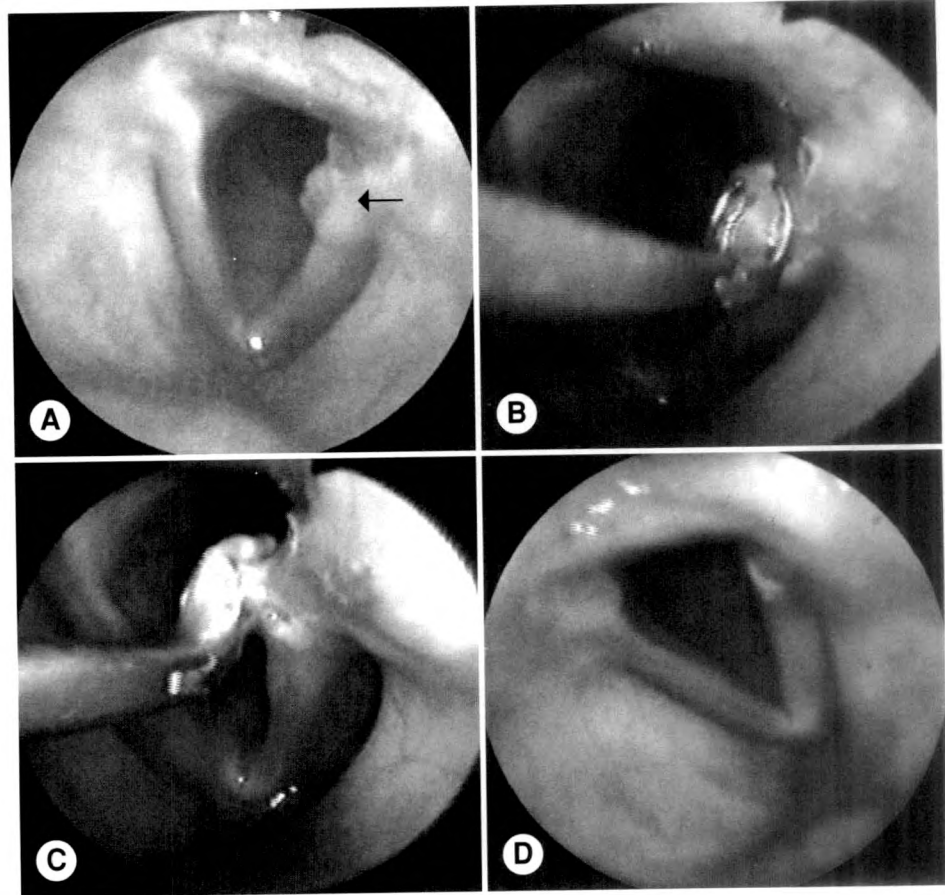
the glottis and no sign of reflux.

Surgical Procedure. The patient is seated during the operation. For topical anesthesia, 4% lidocaine hydrochloride is applied to the mucosal surface of the oral cavity, pharynx, and larynx until the gag reflex disappears. An assistant inserts a fibroscope (ENF T3, Olympus Co, Tokyo, Japan) connected to a charge-coupled device camera system (OTV-S4, Olympus Co) transnasally. A high-resolution laryngeal image is displayed on the monitor. The patient is asked to stick out and hold his or her tongue. Then, the operator manipulates specially developed surgical instruments transorally. The whole system of this surgery is shown in Fig 1. Figure 2 shows specially developed fine-tipped forceps (Nagashima Co, Osaka, Japan), and Fig 3 shows a curved injection needle.

The surgical procedures include excision with forceps, steroid injection, and laser vaporization. The granuloma in Fig 4 was excised with forceps. When the gag reflex is fully suppressed by topical anesthetic, no vocal cord movement occurs during surgery unless patients vocalize voluntarily. Thus, there is no problem for the procedure with vocal cord movement. When the forceps touch the granuloma, the perception of the cartilage of the vocal process is obtained. Careful manipulation can achieve an appropriate depth perception and prevent damage to the perichondrium. In most cases, granulomas can be removed completely with no remaining tag. When tags do remain, additional removal can be easily carried out repeatedly until the tags are completely taken off. There is no concern about dropping the granuloma after grasping it with the forceps, because the forceps have enough power to hold it. Steroid injection can be relatively easily performed with a curved needle (Fig 5). Laser surgery is another alternative in treating granuloma. In this setting, the KTP laser is preferable, because the laser beam is easily shot in any direction transorally through the flexible laser-leading cable (Fig 6).

The patients underwent these procedures, and the surgical outcomes were reviewed in follow-up peri-

Fig 4. Excision of granuloma. **A)** Granuloma on left vocal process is indicated by arrow. **B-D)** Granuloma is grasped with fine-tipped forceps and then removed.



ods from 6 months to 9 years (mean, 42 months).

RESULTS

Intubation Granulomas. One of the 5 patients could not tolerate the operation because of a strong gag reflex. She underwent endoscopic microsurgery under general anesthesia. Three of the other 4 patients underwent excision with forceps alone. In the other patient, steroids were injected after excision. All cases were well controlled with a single procedure and showed no evidence of recurrence of granulomas during follow-up periods from 16 to 72 months.

Hyperfunctional Granulomas. Both patients were

treated with simple excision for the first operation. One has remained free of disease for 18 months. The other demonstrated recurrence 3 months after operation, and underwent excision again. The granuloma has not recurred in 36 months. Voice therapy was not given to these patients, because they could not visit our hospital frequently.

Hyperacidic Granulomas. Of the 21 patients, 9 (43%) demonstrated recurrence of granulomas after the initial procedure. The recurrence rates according to the initial procedures were 38% for simple excision, 33% for excision with steroid injection, and 40% for laser surgery. Repeated procedures were required

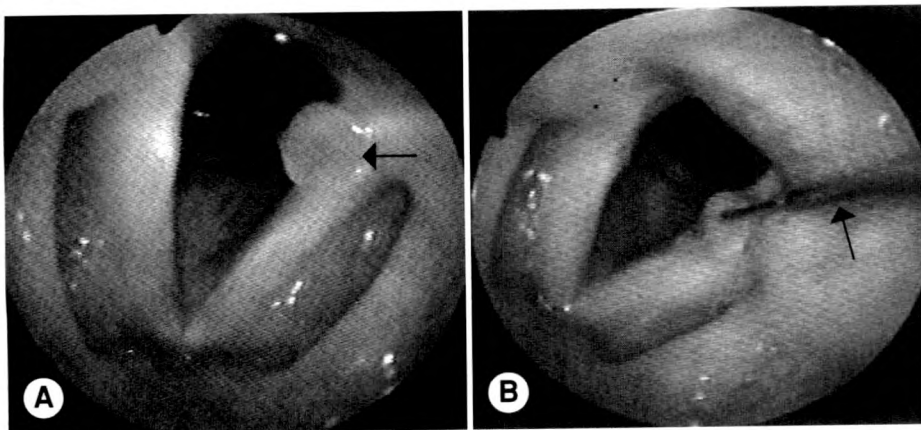


Fig 5. Steroid injection of granuloma. **A)** Granuloma on left vocal process is indicated by arrow. **B)** Steroid is injected into granuloma with curved needle (arrow).

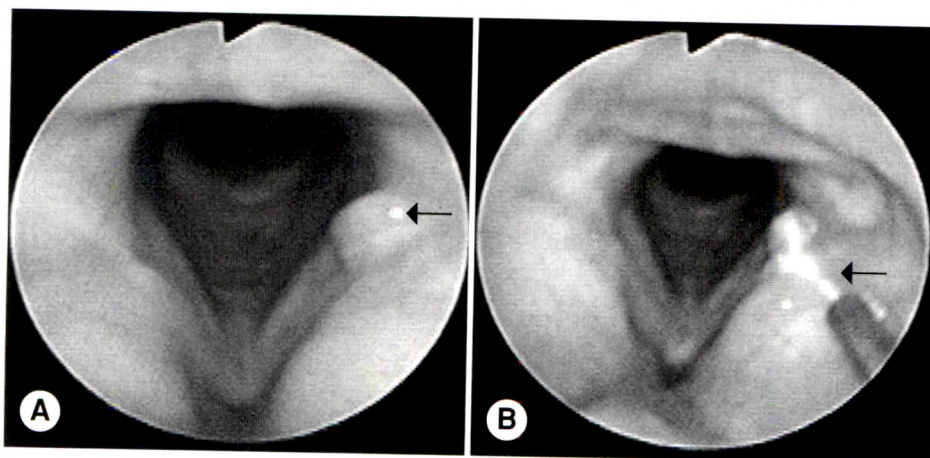


Fig 6. KTP laser surgery. **A)** Arrow indicates granuloma on left vocal process. **B)** Granuloma is vaporized by KTP laser. Arrow — leading cable of KTP laser beam.

to resolve recurrent lesions twice in 3 cases, 3 times in 5 cases, and 9 times in 1 case. Consequently, all but 1 case could be controlled by 3 or fewer operations. No recurrent lesion has been observed during follow-up periods from 6 months to 8 years after the final procedure. As a treatment for reflux, precautions for dietary modification were given to all patients. Proton pump inhibitors (PPIs) were administered to 2 patients with signs of severe reflux after the surgical procedure.

DISCUSSION

Treatment of vocal process granuloma has been controversial because of the high recurrence rates after surgery. Generally, intubation granuloma has been considered a good indication for surgical removal with an excellent outcome.¹ The problem is how to treat contact granulomas in vocal abusers and GER patients. The recurrence rates have been reported to be approximately 30% to 40%.⁸⁻¹⁰ Some authors have reported a 92% recurrence rate following surgery.¹¹ Benjamin and Croxson⁸ reported that 50 operations were required to treat 16 granulomas and that 6 lesions still remained after the final procedure. McFerreran et al⁹ reported the overall mean number of operations to be 2.7 per patient, and the final cure rate to be 68.7%.

To overcome refractory contact granulomas, voice therapy has been conducted for vocal abusers. Bloch et al⁵ stated the success rate of treatment as 71%, or 100% if the patients who terminated voice therapy too early were eliminated. They considered contact granuloma as a contraindication to surgery. However, the outcome of voice therapy might depend upon the ability of the voice therapist, and has not always been optimal. Another disadvantage of voice therapy may be the long period of treatment required. Another alternative is PPIs, especially for contact granulomas caused by GER. Many reports have indicated a good resolution of granuloma with PPIs.^{12,13} The cure rate, however, is still not sufficient. Hanson et al¹² demon-

strated 2 recurrences among 6 cases treated with PPIs. Wani and Woodson¹³ reported that 14 of 18 cases were cured. In some institutions, botulinum toxin type A has been applied to patients with contact granuloma. The botulinum toxin was injected into the thyroarytenoid muscle, inducing paralysis of the muscle and preventing forceful closure of the arytenoid cartilages during phonation. The effectiveness of this method has been reported.¹⁴

Although many conservative treatments have been developed, the treatment period tends to be longer, taking at least a couple of months, and the effectiveness seems to be insufficient. Surgical removal is certainly the quickest therapy, and more than half of the cases were cured by the initial procedure in the present series. Even most recurrent lesions could be controlled by a couple of operations. The reason surgery has been avoided may be that endoscopic phonomicrosurgery under general anesthesia has previously been required to treat each recurrent lesion. Fiberoptic laryngeal surgery under topical anesthesia has facilitated treating refractory granuloma repeatedly. The procedure is much simpler than endoscopic phonomicrosurgery. It can be performed as outpatient surgery. It is possible to combine voice therapy or PPI prescription with this procedure, especially in treating refractory cases. Our usual practice is to remove granuloma by fiberoptic laryngeal surgery with or without other conservative therapies.

One of the most important points in achieving the procedure is the perfect control of the gag reflex. Topical anesthesia was induced with repeated lidocaine spray for all patients in the same manner, and the gag reflex disappeared successfully in all patients but 1. The patient with the remaining gag reflex underwent endoscopic phonomicrosurgery under general anesthesia. The present procedure is considered contraindicated for such a patient.

In this study, we performed 3 different procedures: simple excision with a cold instrument, excision with

steroid injection, and laser vaporization. The recurrence rates showed no apparent difference among the procedures. Laser vaporization has been considered to facilitate healing by avoiding trauma to the arytenoid cartilage and removing abnormal tissues after epithelialization is established.¹⁰ However, the recurrence rate did not seem to be affected by the use of laser in our series, and this finding is compatible with the results reported by Benjamin and Croxson.⁸

The pathological process of granuloma has been proven to involve the arytenoid cartilage, as well as the epithelium. Benjamin and Roche¹⁵ demonstrated osteosclerosis of the arytenoid cartilage using computed tomography in 21 patients with granulomas. They speculated that the osteosclerosis was likely to be secondary to reactive hyperemic and infective

changes in the perichondrium at the base of the granuloma. Surgical removal of the granuloma does not treat all of the local pathological process. However, repeated removal may contribute to prevention of recurrence by inducing scar on the epithelium of the arytenoid cartilage.

CONCLUSION

We developed a minimally invasive technique of laryngeal surgery with fiberoptic monitoring under topical anesthesia for treating vocal process granulomas. This method can be carried out repeatedly and relatively easily, and thus is suitable for recurrent granulomas. In the present series, all lesions were resolved by this method. We believe that surgical intervention is useful in treating vocal process granuloma.

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GAMMA PROBE ACCURACY IN DETECTING THE SENTINEL LYMPH NODE IN CLINICALLY N0 SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

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The sentinel node, which is not always located at the first-echelon nodal area close to the primary tumor, is the initial recipient of possible metastasis. In order to verify the usefulness of the sentinel lymph node technique in the staging of head and neck cancers, we studied 31 untreated patients with squamous cell carcinoma of the upper airways by injecting them around the primary tumor with technetium 99m albumin microcolloid (^{99m}Tc) and submitting them to selective neck dissection with en bloc tumor removal. The nodes were examined after γ -probe identification of the sentinel node(s). No patients were found to have microscopic tumor spread outside the level(s) containing the γ -probe-identified sentinel node(s). In only 1 case was the metastatic node not the γ -probe-positive one, but an adjacent one. Lymphoscintigraphy with ^{99m}Tc seems reliable in identifying the sentinel node(s) and might contribute to the reduction of surgical morbidity by reserving therapeutic neck dissection for cases with histologically confirmed nodal metastasis.

KEY WORDS — gamma probe, head and neck carcinoma, lymphoscintigraphy, sentinel node.

Controversy still exists about the surgical treatment of clinically N0 patients with primary head and neck squamous cell carcinoma (HNSCC). The use of tumor-related histopathologic factors in predicting lymph node metastases (site and class of T, grade, growth pattern, thickness, perineural infiltration, and others)¹⁻⁵ is not a completely reliable method, as demonstrated by the possible presence of metastases from an occult tumor and by the frequency of metastatic lymph node(s) (pN+) in superficial extending carcinoma of the hypopharynx and larynx.^{6,7}

One choice may be no treatment of the neck, if the risk of pN+ is less than a set percentage (15% to 20%). There are some doubts about the effectiveness of this policy: it would mean that a non-negligible number of patients are candidates for late development of lymph node metastasis, which is more difficult to treat, compared with the elective treatment of an occult metastasis. A second choice may be systematic dissection of the lymph nodes. During the past few decades, the surgical approach to elective neck dissection has substantially improved: from radical neck dissection to modified radical dissection and finally to selective dissection. Nevertheless, even in selective dissection, some morbidity still exists, and one must consider that on the whole, two thirds of

N0 necks are also pN0.⁸

The sentinel lymph node is a node that receives drainage directly from the tumor site; it is therefore the initial possible recipient of metastatic tumor cells and may predict the histopathologic status of the remaining lymphatic area.⁹

The sentinel node technique is a reliable tool in the staging of melanoma and breast carcinoma. Recently published reports suggest that this technique may also be useful in deciding the treatment of the N0 neck.⁹⁻¹³

A problem similar to the treatment of the N0 neck is the treatment of clinically negative contralateral lymph nodes when metastases from a primary paramedian tumor are already present in the homolateral nodes.

The aim of this study was to verify whether lymphoscintigraphy and the use of a γ -probe would be a reliable tool in identifying the sentinel node in a homogeneous series of previously untreated HNSCCs.

MATERIALS AND METHODS

From May 1999 to July 2000, 31 untreated patients with squamous cell carcinoma (SCC) of the

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oral cavity (17 patients), oropharynx (9 patients), larynx (4 patients), or hypopharynx (1 patient) were enrolled in this study without any modification of the ongoing treatment protocols. The patients were 24 men and 7 women with an average age of 64 years (range, 36 to 85 years). After a preoperative evaluation including a computed tomography scan of the whole neck, the tumors were staged as follows: 8 T1, 14 T2, 6 T3, and 3 T4. There were 17 N0 and 14 N+ patients (5 N1, 4 N2a, and 5 N2b). The patients with homolateral metastatic nodes had a paramedian SCC, and the study was carried out on the contralateral, clinically negative lymph nodes. After tracheotomy and the induction of general anesthesia, 0.5 mL of technetium 99m albumin microcolloid (^{99m}Tc ; mean, 37 MBq) was injected at 4 different points, 1 cm in depth, at 0.5 cm from the tumor margin. Subsequently, selective neck dissection was performed on the sites without clinical evidence of nodal metastases (levels 1 to 3 in patients with oral cavity or oropharyngeal tumors, and levels 2 to 4 in patients with laryngeal or hypopharyngeal tumors). A total of 37 selective neck dissections were evaluated: in the N0 group, 11 patients with lateral tumors had homolateral selective dissection, and 6 patients with median or paramedian tumors had bilateral selective dissection. The 14 N+ patients underwent homolateral dissection of all levels and contralateral selective dissection.

Thread markers were applied to make the subdivision among levels easier. An en bloc resection of the tumor was performed, the specimen was placed on a sterile wrap, and the levels were clearly marked with a pen by the surgeon. The sentinel node(s) were immediately identified by the nuclear medicine specialist and the pathologist with the aid of a γ -probe. The γ -probe used is a handheld γ -detector (Navigator Gamma Guidance System, RMD, Watertown, Massachusetts). The nodes are considered positive if the radioactivity level is at least 4 times the background level.

The pathologist isolated the sentinel node(s) and all of the identifiable remaining lymph nodes and examined them by standard frozen section and/or intraoperative cytology. If the intraoperative pathological examination revealed metastatic cells in 1 or more lymph nodes, the dissection was completed on the remaining levels.

In the last 7 patients, the identification of the sentinel node(s) was performed 20 minutes after the injection with a handheld γ -probe through the intact skin, immediately before the dissection. The results were compared with the subsequent identification on the specimen.

RESULTS

The results are summarized in the Table. In the N0 group, 1 to 5 sentinel lymph nodes (total, 33) were identified in the selective dissections of 15 patients (15/17 with homolateral dissection and 2/6 with contralateral dissection); at histologic examination in 10 patients, there were no metastases in the ^{99m}Tc + nodes, and the remaining nodes also were negative. In 5 patients, metastases were found in 1 or more of the γ -probe-positive nodes (4 cases) or in an adjacent node of the same level (1 case). In 2 patients, lymphoscintigraphy did not identify a sentinel node, and the histologic findings of all of the lymph nodes were negative for metastases.

In the N+ group, 1 to 2 sentinel lymph nodes (total, 12) were identified in the selective dissections of 7 patients; at histologic examination in 5 patients, there were no metastases in the ^{99m}Tc + lymph nodes, and the remaining nodes were also negative. In 2 patients, metastases were found in the γ -probe-positive nodes (1 with a low level of ^{99m}Tc positivity). In 7 patients, lymphoscintigraphy did not identify a sentinel node, and the histologic findings of all of the lymph nodes were negative for metastases.

Worthy of note, in 6 of the 7 cases with metastatic ^{99m}Tc + lymph nodes, histopathologic examination showed neoplastic deposits only in the sentinel nodes, and in 3 cases they were micrometastases. Moreover, no patients had microscopic tumor spread outside the level(s) containing the γ -probe-identified sentinel node(s). Preoperative sentinel lymph node evaluation corresponded to the subsequent identification on the specimen in 5 of the 7 patients examined. In the remaining 2 patients, preoperative ^{99m}Tc positivity was due to extralaryngeal spread of a transglottic tumor (patient 30) and to tumor or muscle uptake (patient 26).

DISCUSSION

Although not with the same frequency reported in melanoma, even in HNSCC we may observe "skip" metastases. Byers et al,¹⁴ among 277 patients with SCC of the mobile tongue, reported that 16% of metastases bypassed the first-echelon nodal area. In our series, 2 N0 patients with SCC of the oral cavity had γ -probe-positive and histologically metastatic nodes: only at the third level in one case (patient 1) and at the second and third levels in the other (patient 11).

Mamelle et al,¹⁵ in a series of 914 patients with tumors of the oral cavity, oropharynx, hypopharynx, or larynx, demonstrated that besides the number of positive nodes, the most significant nodal prognostic factor is the site of the positive node (inside or outside the first-echelon level in relationship to the

CLINICAL AND PATHOLOGICAL CHARACTERISTICS OF TUMORS AND LYMPH NODES

Patient	Site of T	Clinical Stage	Site of Neck Dissection	^{99m} Tc + N Level*	^{99m} Tc + N Histology	N Status (N+/Total N)
17	OC bilat	T1N0	H + C	1/II° L	Negative	0/19 L; 0/16 R
20	OPh bilat	T1N0	H + C	2/III° L	1 Positive micrometastasis; 1 negative	1/29 L; 0/24 R
6	OC bilat	T2N0	H + C	0		0/18 L; 0/15 R
16	OC bilat	T3N0	H + C	2/II° R	Negative	2/33 L; 0/34 R
30	Lar bilat	T4N0	H + C	1/III° L; 1/IV° L	Negative	0/26 L; 0/9 R
31	OC L	T2N0	H + C	4/II° L; 1/III° L	Negative	0/46 L; 0/20 R
4	OC R	T1N0	H	2/II° R	Negative	0/22 L
8	OC L	T1N0	H	1/I° L; 1/II° L	Negative	0/27 L
22	OC R	T1(m)N0	H	1/I° R; 1/II° R	Negative	0/25 R
1	OC L	T2N0	H	1/III° L	Positive micrometastasis	1/45 L
2	OC R	T2N0	H	1/I° R; 2/III° R	Negative	0/29 R
5	OPh L	T2N0	H	1/I° L	Negative; positive adjacent N of same level	1/31 L
11	OC R	T2N0	H	1/II° R; 1/III° R	Positive	2/9 R
19	OC R	T2N0	H	1/I° R; 1/II° R	Negative	0/47 R
27	Lar R	T2N0	H	0		0/27 R
25	OC L	T3N0	H	2/II° L	Negative	0/23 L
24	OC L	T4N0	H	1/II° L	Negative	0/37 L
7	OPh R	T1N1	C	0		0/27 L
23	OC bilat	T1N2a	C	1/I° L	Negative	0/35 L
9	OPh R	T1N2a	C	0		0/22 L
13	OC R	T2N1	C	1/I° L; 1/II° L	Negative	0/22 L
18	Lar R	T2N1	C	1/II° L	Negative	0/13 L
3	OPh R	T2N2a	C	2/III° L	Negative	0/53 L
29	OPh L	T2N2a	C	1/II° R	Negative	0/24 R
12	OPh L	T2N2b	C	1/II° R	Negative	0/16 R
26	OC R	T2N2b	C	0		0/30 L
14	Lar bilat	T3N1	C	0		0/32 L
15	HPh L	T3N1	C	0		0/19 R
10	OPh R	T3N2b	C	0		0/22 L
21	OC R	T3N2b	C	1/II° L	Positive (low γ-probe uptake) micrometastasis	1/25 L
28	OPh L	T4N2b	C	2/II° R	1 Positive; 1 negative	4/16 R

OC — oral cavity; H — homolateral; C — contralateral; OPh — oropharynx; Lar — larynx; HPh — hypopharynx.

*Arabic numeral is number of ^{99m}Tc-positive nodes detected. Roman numeral is level at which nodes were located.

site of the primary tumor).

After some unsatisfactory reports on the use of blue dye to detect the sentinel node during operation,^{8,12} more recent studies used ^{99m}Tc. Koch *et al.*,¹¹ in a series of 5 N0 patients, identified a sentinel node in 2 (metastatic cells were demonstrated in 1) and were unable to identify the sentinel node in 3 (2 of whom had metastatic nodal disease). Shoaib *et al.*¹² identified sentinel nodes in 15 of 16 patients, and the biopsy was accurate in the N0 necks that contained impalpable metastases. Zitsch *et al.*¹³ identified the sentinel node in each of 8 N0 patients; 4 of these patients were pN+ (2 with metastases only in the sentinel node and 2 both in the sentinel node and in different nodes). Also, Alex *et al.*⁹ identified the sentinel nodes in each of 8 N0 patients, 1 of whom was pN+; in no instance

was a sentinel node negative for micrometastatic disease while a nonsentinel node was positive.

To date, this is the largest published series concerning the use of lymphoscintigraphy in identifying the sentinel node in HNSCC, and our results further confirm the reliability of this technique. Nevertheless, some problems remain.

1. A high radioactivity level in the tumor area may mask the presence of a sentinel node or may simulate it, particularly in oropharyngeal and laryngeal neoplasms, because of the nearness of the tumor site to the draining lymph nodes. In these cases, a lead plate may be used as a shield between the tumor and the nodal site, and/or the tip of the handheld probe may be directed to avoid the alignment of the tip of the probe, the N area, and the tumor.

2. The threshold level of radioactivity compared to the background noise has not yet been fully assessed in defining a lymph node as "sentinel," either through the skin or on the specimen. Moreover, these data should always be specified, to allow a comparison among the results of different series.

3. The handheld γ -probe is not as yet widely available. A γ -camera to detect the sentinel node(s) after the injection of the ^{99m}Tc is available in the large majority of hospitals. The injection may be done either in the γ -camera room, without general anesthesia, for oral cavity tumors, or in the operating room (transporting the patient thereafter to the γ -camera room) for oropharyngeal and laryngeal tumors. The study may be performed the day before the operation, and the results may be discussed with the patient, ie, the type of skin incision and the type of nodal dissection.

4. The tracer ^{99m}Tc was chosen because it is easily available and because its large sulfur colloid particles remain within the sentinel nodes for several hours, without diffusion to more distant and nonsignificant nodes. Some authors^{13,16} do not agree with this hypothesis on the biokinetics of the tracer and suggest the use of intermediate-sized colloidal albumin as a carrier for the technetium. Moreover, we do not know whether it is reliable to inject and examine the patient only in the supine position, ignoring the possible influence of the physiologic gravity force on the lymphatic flow.

5. The large number of false-positive results may be explained by the fact that ^{99m}Tc is an inert substance that must passively migrate from the injection site to the lymph nodes, whereas the neoplastic cells are alive and active and follow the same path whenever they migrate.

6. The detection of the sentinel node is a preoperative determination of the pathway of cancer spread. Its ultimate aim is to decrease the number of nontherapeutic neck dissections without increasing the oncological risk. While we await further improvement of this investigation technique, the test, if positive, indicates where to perform the lymph node biopsy. All of these patients are candidates for neck dissection. If the surgical plan is to be based in future on the use of this test, the biopsy of the sentinel node will indicate whether a complete dissection is really necessary. Therefore, a false-positive result would determine only a lymph node biopsy, instead of a dis-

section.

7. There is no agreement about the pathological examination of the sentinel node, particularly with regard to the accuracy of intraoperative evaluation, the number of histologic sections to be examined, and the use of ancillary techniques for the identification of micrometastases. In order to achieve a higher diagnostic accuracy, serial histologic sections and immunohistochemistry can be used. On the other hand, the standard, simple, and cheaper histopathologic examination may be reliable in assessing the nodal status. Over the past 10 years, we have used the conventional examination technique (2 histologic sections at different levels for each identified node), with 437 lymph node dissection specimens obtained from 326 patients with HNSCC pathologically staged as N0. (Of these patients, 163 received postoperative radiotherapy for reasons related to the contralateral neck side or to the primary tumor.) Only 1 recurrence (0.2%) was observed on the N0 side in these patients (unpublished data).

8. There are not sufficient data to decide whether to sample only the γ -probe-identified node(s), or to remove all of the nodes within the same level (thus performing a "superselective" γ -probe-guided neck dissection).

CONCLUSIONS

Our data confirm that in N0 HNSCC, as in malignant melanoma and breast cancer, if the sentinel node is free of metastasis or there are no sentinel nodes, finding metastases elsewhere in the tumor-draining lymphatic area is very unlikely. The clinical experience here reported, and the more recently published studies, indicate that lymphoscintigraphy with ^{99m}Tc may be a reliable tool in identifying the sentinel node, provided that an improved and standardized detection procedure is defined. It may contribute to a less aggressive and more patient-oriented approach, reducing the morbidity of the lymphatic sampling without compromising its oncological benefits, and thus reserving therapeutic neck dissection only for patients with histologically confirmed nodal metastases.

Further large studies and adequate follow-up are necessary to verify whether neck dissection in the N0 neck after γ -probe identification and histopathologic examination of the sentinel node (or of the level containing the sentinel node) can be avoided, and if so, whether this procedure can be deemed oncologically safe.

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ISOLATED CONGENITAL ROUND WINDOW ABSENCE

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Absence of the round window as a congenital defect is a rare entity that causes a conductive hearing loss. To the best of our knowledge, isolated and nonsyndromal round window absence has only been reported 3 times; the diagnoses were made during surgery. We are reporting on another case that was diagnosed before operation by virtual endoscopy of the middle ear during a high-resolution computed tomography scan. Although this new technique requires further validation, it may be an alternative to surgery as a means of confirming this rare diagnosis.

KEY WORDS — congenital conductive hearing loss, round window absence, virtual endoscopy.

INTRODUCTION

Absence of the round window is a rare congenital defect that has been reported in syndromal or isolated cases.¹⁻⁷ In syndromal forms, it is associated with mandibulofacial dysostosis (Franceschetti syndrome),¹ endemic cretinism,² ossicular malformation,^{1,3} or stapes ankylosis.^{3,4} To the best of our knowledge, isolated and nonsyndromal round window absence has only been reported 3 times.⁵⁻⁷ We report a new case of an isolated bilateral congenital absence of the round window diagnosed by a high-resolution computed tomography (CT) scan of the middle ear with virtual endoscopy.

CASE REPORT

A 25-year-old woman with no family history of hearing loss or personal history of otitis presented with a lifelong complaint of bilateral hearing loss with intermittent tinnitus. The physical examination showed normal external ears and tympanic membranes. Bone conduction was bilaterally greater than air conduction by Rinne examination. Weber testing was lateralized to the left ear. The audiological studies demonstrated a bilateral and asymmetric conductive hearing loss (Fig 1), type A tympanograms, and absent acoustic reflexes. A high-resolution CT scan of the temporal bone showed no ossicular malfor-

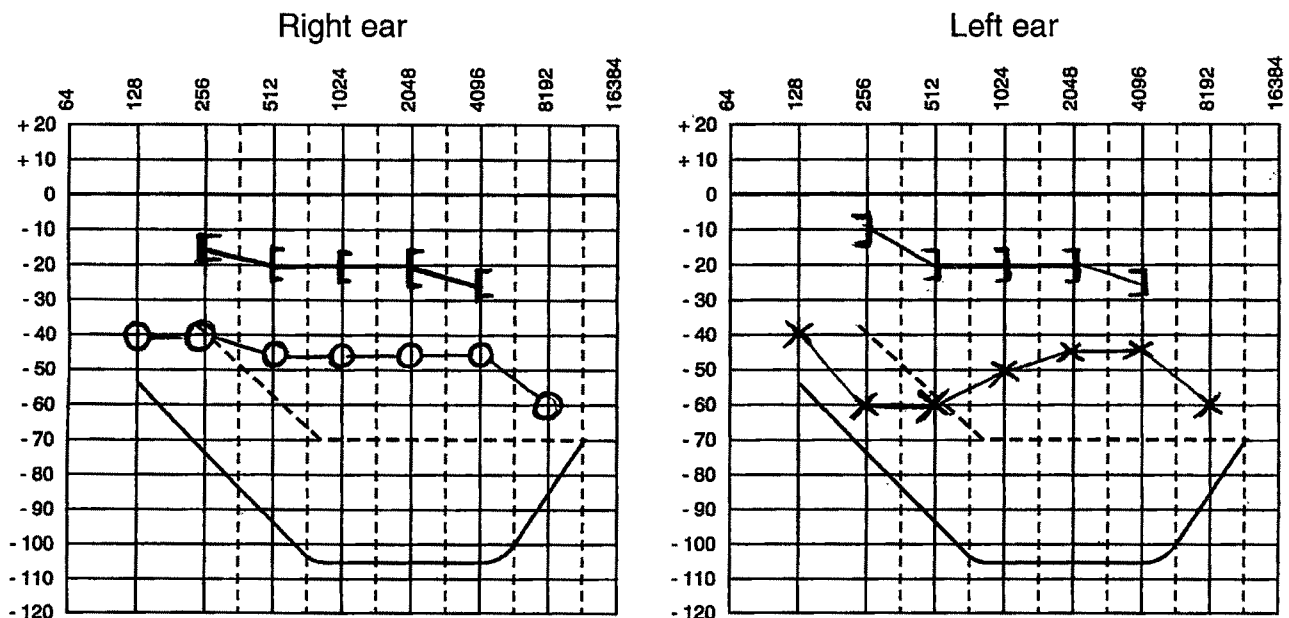


Fig 1. Preoperative audiograms.

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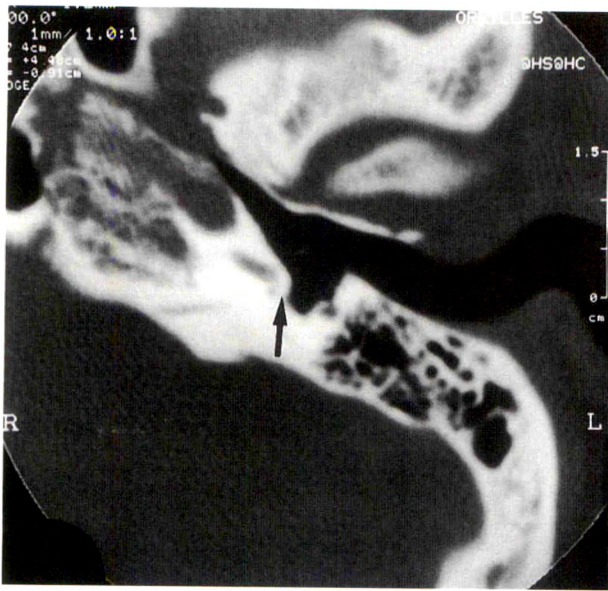


Fig 2. Axial high-resolution computed tomography scan of left temporal bone. Round window niche is absent (arrow). Contralateral image was identical.

mation and no bone resorption indicative of otospongiosis. The axial views of the CT scan for the left middle ear seemed to indicate an absence of the round window (Fig 2), which was more evident on the virtual endoscopic image (Fig 3A). The axial CT scan and the virtual endoscopic image of the right middle ear appeared identical. After informed consent, the patient underwent left middle ear exploration to confirm this rare diagnosis and to search for an associated stapes ankylosis. The round window was absent (Fig 4), and the ossicles and stapes footplate were mobile and normal. Consequently, no further surgery was performed, and the postoperative audiogram showed no change.

DISCUSSION

A description of the round window was first published in 1772 by Antonio Scarpa.⁸ Embryologically,

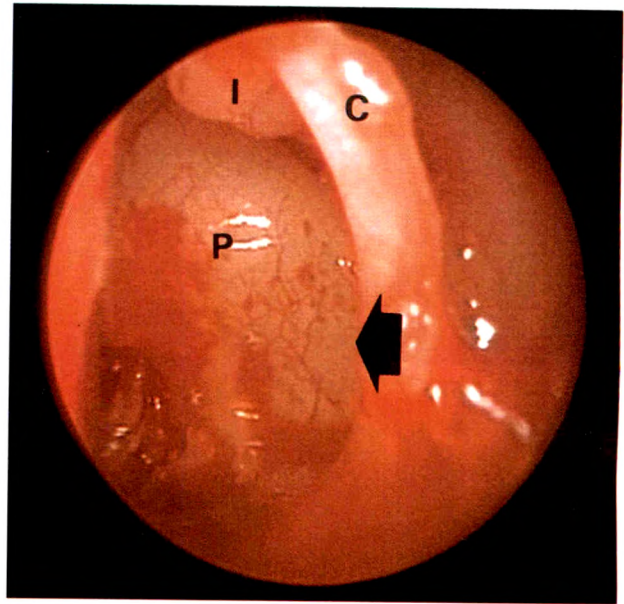


Fig 4. Surgical view of left middle ear shows absence of round window niche (arrow) and shows promontory (P), incudostapedial articulation (I), and chorda tympani nerve (C).

the round window area is recognized during the 11th week of gestation as a condensation of embryonic connective tissue between the scala tympani and the fossula of the cochlear fenestra. During ossification of the otic capsule, a cartilage ring located in the round window niche prevents ossification of its opening. However, if the cartilage ring fails to develop, the round window may be absent.^{3,9}

Congenital obstruction of the round window causes a conductive hearing loss either unilaterally⁷ or bilaterally,^{5,6} as in this case. The mechanism of the conductive hearing loss can be explained if the labyrinth is considered as a hydraulic system. One of the functions of the round window membrane is to release the mechanical energy transmitted to the labyrinthine fluid from the stapes footplate.^{3,10} As this fluid is noncompressible, rigid closure of the round window

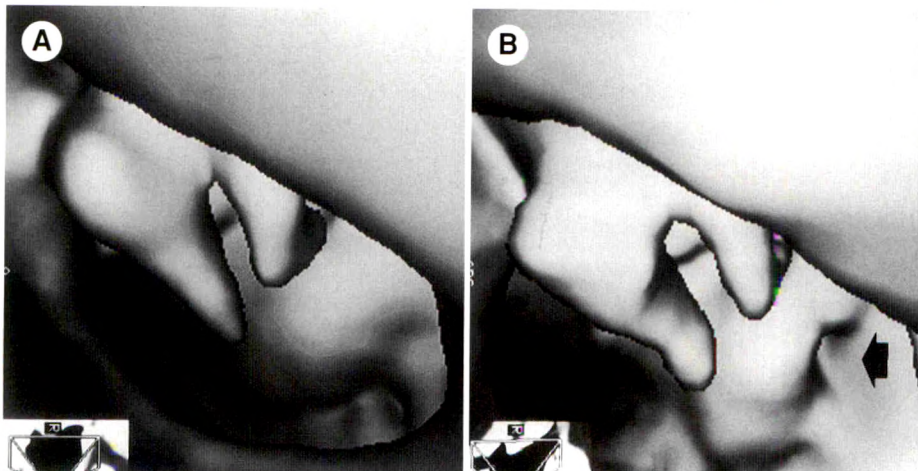


Fig 3. Virtual endoscopy of left middle ears. A) Absent round window niche. Contralateral image was identical. B) Control; 25-year-old woman with normal round window niche (arrow).

niche by bone limits movement of the footplate in the oval window. Reduced compliance of the sound conduction mechanism results in a conductive hearing loss.

In the literature, isolated round window absence has only been reported 3 times, and was diagnosed during surgical exploration without a preoperative CT scan.⁵⁻⁷ In 1 case, a postoperative CT scan suggested the absence of the round window on axial and coronal views.⁷ We are reporting the first case to be diagnosed before operation on a CT scan with virtual endoscopy of the middle ear. A high-resolution CT scan with axial and coronal views, with cuts obtained at 1-mm intervals rather than 1.5 mm, suggested the diagnosis.⁴ However, we believe that virtual endoscopy using not 2-dimensional, but 3-dimensional, reconstructed views is more reliable in demonstrating this anomaly.¹¹⁻¹⁴ This new software provides an accurate visualization of the round window fossa^{11,13} very similar to that obtained during surgical exploration (Figs 3A and 4). In this case, comparison between a pathological view (Fig 3A) and a normal view (Fig 3B) is particularly demonstrative. Although this new software tool requires fur-

ther validation, it should be performed before operation when a round window absence is suspected, and it could be an alternative to surgery in confirming this rare diagnosis.

A surgical treatment was initially reported, but is considered rather controversial.⁴⁻⁷ Its goal was essentially to confirm the absence of the round window and to eliminate an associated stapes ankylosis.⁴ However, if the round window is absent, a fenestration of the promontory should not be performed because of a high risk of deafness. When absence of the round window is associated with a stapes fixation, a stapedectomy gives variable results with a constant risk of hearing loss and vertigo.⁴ Although surgery is a reliable tool for confirming the diagnosis, it has little chance to improve the patient's hearing loss.

In conclusion, isolated congenital round window absence can cause a conductive hearing loss. The diagnosis may be done without surgery by careful analysis of the round window niche on a high-resolution CT scan with virtual endoscopy. Although this use of virtual endoscopy requires further validation, we believe that it could replace the surgical exploration.

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RECEPTIVE AND EXPRESSIVE LANGUAGE SKILLS OF CHILDREN WITH FIVE YEARS OF EXPERIENCE USING A COCHLEAR IMPLANT

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The language of 7 children who had used a cochlear implant for 5 years was evaluated by means of the Clinical Evaluation of Language Fundamentals-3, the Peabody Picture Vocabulary Test-Revised, and the Expressive Vocabulary Subtest of the Test of Word Knowledge. All subjects demonstrated impaired skills relative to normal-hearing children on 1 or more subtests. Variability in performance occurred between subjects and within subjects across subtests. Strengths in semantic skills were evident compared to weaker syntactic and morphological abilities. The nature of the subjects' language impairments is considered with reference to language delay and specific language impairment. The findings support the need for further evaluation of the rate and course of development of language subskills. Investigation of the influences of information processing, and learning styles and strategies, on children's outcomes is also warranted. These findings have implications for implementation of optimal habilitation and education programs for children with cochlear implants.

KEY WORDS — child, cochlear implant, language.

INTRODUCTION

The ultimate goal of cochlear implantation for children is to provide sufficient hearing to enable use of audition for speech and language development.¹ For a proportion of children, this goal is being met.²⁻⁸ However, it has been commented that giving cochlear implants to children, particularly those who are prelingually deaf, may not provide adequate hearing for them to acquire skills that allow effective participation in the hearing world.⁹ It is suggested that their ability to fully belong in the hearing world or the deaf world may be compromised.⁹

Speech perception outcomes show that many children receive better sensory assistance from a cochlear implant than from other sensory aids.^{3,10-12} In turn, improved speech perception skills appear to support improved speech production.¹³ However, research examining speech perception and production outcomes does not address the second part of the goal, namely, that children with an implant will be able to use audition for effective language development. Recently, a greater focus on evaluating language development in children with cochlear implants has emerged.

One approach has been to compare the rate of language development of children with a cochlear implant to that of children with normal hearing. Comparisons between the groups are based on pairing children of the same language age at the time of implantation, regardless of chronological age, and eval-

uating changes in their language age over time. For children with a profound hearing loss who use hearing aids, it is commonly reported that the gap between language age and chronological age increases with time.^{2,14} In contrast, the rate of language development of some children after cochlear implantation has been shown to increase to a rate similar to and sometimes exceeding that of children with normal hearing. This has enabled the gap between their language age and chronological age to remain constant or narrow to some extent.^{2,4,6,8,12,15-17} However, one report stated that the rate of receptive language development slowed in the second year after implantation, suggesting that this rate may not be sustained.⁴ Additionally, these results of enhanced rates of development were not consistent across all children; several researchers observed vast differences between subjects.^{2,8,15} Despite an enhanced overall rate of language acquisition in some children with a cochlear implant, as compared to children of the same age with normal hearing, many children with an implant continue to have significantly impaired language skills.^{1,2,4,6,8,15}

From these data, it could be concluded that children who use cochlear implants have a delay in their language development and that their skills are similar to, and appropriate for, a child with normal hearing of a younger age. This brings an expectation that in time the language of children with an implant may catch up with, or come close to, that of their hearing peers.^{4,8,14}

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The suggestion that the language of children with a cochlear implant may progress toward that of their hearing peers has generally been made in regard to results from language assessments of very broad measures of general language achievement.^{4,8,14} It is well known, however, that effective language use requires successful development and integration of a range of language subskills. One report commented on the need for children with an implant to achieve "successful orchestration" of all 5 rule systems of communication, including pragmatics, semantics, syntax, morphology, and phonology.¹⁸ Therefore, comprehensive evaluation of each of these areas was identified as an important issue in measuring communication outcomes.¹⁸ Identification of "normal language skills" cannot be claimed without age-appropriate performance across these different skills.

The few investigations of these language subskills in children with a cochlear implant have reported that after implantation, some aspects of language are enhanced more than others.^{19,20} In one study, receptive and expressive vocabulary and expressive concepts were enhanced, but comprehension of syntax and morphology developed at a slower rate.¹⁹ When the morphology and syntax of 6 children with implants was studied over 2 years, each child showed progress, but the rate of progress varied among children. Comparisons to other subskills were not made.²¹ In a further study, children with a cochlear implant showed more strength in vocabulary skills than in morphological development and a preference for using content words over function words.²⁰ Within their morphological skills, there was also differential progress in different areas of the morphological system.²⁰ The important observation was made that good vocabulary development is not sufficient for rapid grammatical development to occur.²⁰

Although many children with a cochlear implant may have more advanced linguistic skills than they would have had without an implant, they may demonstrate specific strengths and weaknesses in different language subskills. This is a frequent observation, not only in children with a hearing impairment (with or without a cochlear implant), but also in children with a range of other diagnoses, including intellectual impairment, blindness, autism, and specific language impairment (SLI).²² The language profiles of these populations are more complex than a generalized delay in development. Appreciating these complexities has significant implications for supporting the communication development of these children. Likewise, consideration of the complexity of the linguistic profile of children with a cochlear implant is critical to our ability to offer effective language habilitation services.

Knowing what factors have an impact upon the

language achievements of children with a cochlear implant is critical to preimplantation and postimplantation planning and decision-making. Variability in achievements on a range of skills, such as speech perception, speech production, and language, is a recurring theme in the literature.^{1-3,8,21} This variability has been attributed to factors such as length of auditory deprivation,^{5,23} age at implantation,^{5,23,24} duration of implant use,^{1,24} communication mode,^{5,15,22,25} and social and family environment,^{5,7} among others.

When considering the variables that might directly affect language outcomes, some researchers have considered the relationship between speech perception and language performance.^{1,3,7,15,25} For children identified as "stars" of implant use (the top 20% of 160 children assessed with the Phonetically Balanced Kindergarten [PBK] Word Test), a strong relationship between speech perception and language abilities was reported. This same relationship was not evident for the controls, chosen from the bottom 20% of the sample.²⁵ A study of a group of children from the Moog Oral School, known for its individualized and well-resourced educational program, also showed that children with higher scores on open-set speech perception with the PBK Word Test had language quotients within the average range for children with normal hearing.⁷ Poorer performance on the PBK Word Test correlated with language quotients below the average range. In contrast to these two studies, when less "select" subject groups have been evaluated, the relationship between speech perception and language skills has been less well defined.^{3,15}

Despite these efforts to elucidate the impact of the complex interplay of variables on communication competence, predictive statements about specific factors affecting outcomes with a cochlear implant for individual children cannot be made with complete confidence. One researcher suggested that the causes of the variability might also include more specific and complex variables. Information processing skills, such as the encoding, storage, retrieval, and processing of spoken language, were identified as possibly being significant in understanding the variability across children with a cochlear implant.²⁵

There remains an extensive and complex range of questions about the language development of children with a cochlear implant. Among these is the question of whether the reportedly rapid rate of language progress in the early years after implantation is sustained. If this is so, is it the case for all children? If not, which children, in what circumstances, are more likely to achieve a normal or faster-than-normal rate of development? Finally, when individual language subskills are considered relative to each oth-

er, is this same effect observed and maintained consistently across the range of skills over time?

This current study aimed to contribute to the discussion around these questions by comparing the language outcomes of a group of children who have used a cochlear implant for 5 years with those of children of the same age who have normal hearing. After 5 years of implantation, one would expect that patterns in long-term language skill levels would have emerged and specific strengths and weaknesses in language subskills might be evident. It is hoped that the findings of this study will contribute important information to our knowledge of the long-term communication outcomes of children with cochlear implants and assist in determining appropriate therapy and educational programs.

METHODS

Subjects. At the commencement of this research project in 1998, all 24 children involved with the cochlear implant team of a major metropolitan hospital were invited to participate in a 2-year study of language outcomes in children with a cochlear implant. For the substudy reported here, 12 of these 24 children had been using an implant for at least 5 years. Of these 12 children, 7 participated in the study. The remaining 5 individuals were not included in the study for a range of reasons. The skill level of 2 children was not adequate for use of the Clinical Evaluation of Language Fundamentals–3rd Edition (CELF-3). One child made limited gains with the implant, in the presence of general cognitive deficits, and parental concern about this outcome may have affected the decision not to participate. Another postlingually deaf child declined participation, possibly because of having limited contact with the cochlear implant team (because of high levels of achievement, with limited support being needed or requested). The final child had already completed a 5-year postimplant assessment with an assessment tool that was not consistent with the tools used in this study (Clinical Evaluation of Language Fundamentals–Revised).

Language Assessment. The language skills of the 7 subjects described in this paper were assessed with the CELF-3, a structured assessment consisting of 4 receptive language subtests and 4 expressive language subtests.²⁶ The subtests administered are determined by the child's age, with 3 receptive and 3 expressive subtests constituting the main test battery at any given age. Six of the 7 subjects were also administered the Peabody Picture Vocabulary Test–Revised, Form M (PPVT-R),²⁷ a measure of receptive vocabulary, and the Expressive Vocabulary Subtest

of the Test of Word Knowledge (TOWK).²⁸ Each of these tests were standardized on children with normal hearing.

Cognitive Assessment. Performance IQ and verbal IQ scores were determined with the Wechsler Intelligence Scale for Children–3rd Edition (WISC-III).²⁹

Speech Perception Assessment. The Northwestern University–Children's Perception of Speech (NU-CHIPS)³⁰ test was used to assess closed-set words. The Bamford-Kowal-Bench/Australian Version Sentence Lists for Hearing Impaired Children (BKB/A Sentences)³¹ were used to assess open-set sentences. The Arthur Boothroyd Word Lists (AB Word Lists)³² were used to assess open-set words. Subjects whose speech intelligibility was poor were not scored on standardized speech perception tests of open-set sentences.

Procedures. Either the language assessments were administered during the research period, or data were collated from results of tests completed before the commencement of the study. Subjects were individually evaluated in a quiet room by a speech pathologist with experience working with children with a cochlear implant. Test responses were recorded at the time of assessment; however, each assessment was videotaped to enable review of responses if required. Cognitive assessments were all completed during the research period. Language and cognitive assessments were administered in the subjects' preferred communication mode of either oral communication or total communication. A qualified interpreter, accessed through the Queensland Deaf Society, interpreted using signed English for children who used total communication.

The results from the subjects' speech perception assessments that were administered closest to 5 years after implantation were collected from their medical charts. For all tests except subject 1's open-set word results, these assessments occurred no more than 6 months before or 6 months after their 5-year postimplantation language assessment. The data reported for subject 1's open-set word scores were collected at 6 years 6 months after implantation and must be viewed in this light.

Data Analysis. Standard scores were calculated for each language subtest for each subject. A mean standard score was calculated for each subtest for all subjects. Standard score results were also expressed as standard deviation units (SDUs) from the norm. An SDU below zero indicates a score that is below average for age. An SDU above 0 indicates a score that is above average for age. At the extremes, ap-

TABLE 1. DEMOGRAPHIC AND MEDICAL HISTORY DETAILS AT FIVE YEARS AFTER COCHLEAR IMPLANTATION

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7
Onset of hearing impairment	Congenital	Congenital	Congenital	1 y 5 mo	Congenital	Congenital	Congenital
Cause of hearing impairment	Unknown	Unknown	Mondini's malformation	Meningitis	Ototoxic medication at 31 wk gestation	Unknown	Unknown
Age at implantation	6 y 10 mo	3 y 1 mo	4 y 4 mo	2 y 3 mo	2 y 6 mo	3 y	4 y 2 mo; 10 y 2 mo*
Type of implant	Spectra 22	Spectra 22	Spectra 22	Spectra 22	Spectra 22	Spectra 22	Spectra 22; Sprint
Active electrodes	20	20	17	20	20	20	20
Mode of map	Common ground	Common ground	Bipolar + 4	Common ground	Common ground	Common ground	Common ground
Communication mode							
Before implantation	TC then OC	OC	OC + some signs	OC	OC	OC	OC
After implantation	OC	OC, TC school	OC	OC, TC school	OC	OC, TC school	OC, then TC
Education							
Primary	MS + AVT	HIU + INT	MS + AVT	HIU + INT, then MS	MS + AVT	HIU + INT	HIU
Secondary	MS + HIU						
Closed-set speech perception 5 years after implantation							
AA Words	100%	NA	100%	NA	NA	NA	100%
Open-set speech perception 5 years after implantation							
AV Sentences	98%	100%	CNS	90%	84%	84%	CNS
AA Sentences	72%	92%	CNS	78%	84%	84%	CNS
AV Words	100%	100%	63%†	100%	100%	97%	80%†
AA Words	79%	96%	60%†	88%	100%	93%	80%†
WISC-III Cognitive Assessments (mean = 100; SD = 15)							
WISC-III PIQ	126	115	103	87	112	113	70
WISC-III VIQ	89	64	87	82	69	67	46
PIQ-VIQ difference	37	51	16	5	43	46	24

TC — total communication, OC — oral communication, MS — mainstream, AVT — advisory visiting teacher, HIU — hearing-impaired unit, INT — some integration into mainstream classes, AA — audition alone, NA — not administered, AV — audition + visual, CNS — could not score because of severe speech disorder, PIQ — performance IQ, VIQ — verbal IQ.

*Second implantation was due to device failure.

†Score may reflect speech disorder, rather than speech perception.

proximately 2.5% of the normal-hearing population would have an SDU of -1.96 or lower or $+1.96$ or higher.

RESULTS

The hearing, communication, education, speech perception, and IQ data of the 7 subjects are detailed in Table 1. In the sample, there were 2 boys and 5 girls. At the time of their 5-year postimplantation assessment, the children ranged in age from 7 years 3 months to 12 years 0 months.

The CELF-3, PPVT-R, and TOWK Expressive Vocabulary Subtest results are shown in Table 2. We calculated SDUs for 6 of the 8 CELF-3 subtests. For the remaining 2 subtests (Sentence Assembly and Semantic Relationships), SDUs were not calculated, as only 2 subjects completed these subtests. Variable

language skills were noted across the group. On 5 of the 6 CELF-3 subtests in which SDUs were calculated, the results were 1.0 SDU or more below the norm mean, which is in the range of the lowest quintile of performance expected in a hearing population. For the other subtest (Word Classes), the result was -0.9 SDU from the norm mean. The subjects had greatest difficulties on the Concepts and Directions, Recalling Sentences, Word Structure, and Formulating Sentences subtests. Their average performances on these subtests equated to the lowest 3.5% of performances expected in the normal-hearing population.

CELF-3 Receptive Language Score and Subtest Performances. The receptive language scores, derived from a combination of each of the receptive language subtest scores, were below the average range

TABLE 2. STANDARDIZED LANGUAGE ASSESSMENT RESULTS AT FIVE YEARS AFTER COCHLEAR IMPLANTATION

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Mean Standard Score (min, max)	Mean Standard Deviation Units (min, max)
Age at assessment	12 y	8 y 5 mo	6 y 10 mo	7 y 3 mo	7 y 11 mo	8 y 5 mo	9 y 5 mo	8 y 7 mo (6 y 10 mo; 12 y)	
CELF-3 Receptive Language Subtests*									
Sentence Structure	NA	4	8	3	6	5	NA	5.2 (3, 8)	-1.6 (-2.3, -0.7)
Concepts and Directions	3	5	6	3	6	3	3	4.1 (3, 6)	-2.0 (-2.3, -1.3)
Word Classes	4	8	12	8	8	8	3	7.3 (3, 12)	-0.9 (-2.3, 0.7)
Semantic Relationships	7	NA	NA	NA	NA	NA	6	7.3 (6, 7)	NC
Receptive Language Score†	61	72	92	61	80	69	53	69.7 (53, 92)	-2.0 (-3.1, -0.5)
CELF-3 Expressive Language Subtests*									
Word Structure	NA	5	4	3	8	3	NA	4.6 (3, 8)	-1.8 (-2.3, -0.7)
Formulating Sentences	3	4	6	6	6	4	3	4.6 (3, 6)	-1.8 (-2.3, -1.3)
Recalling Sentences	3	3	3	3	7	3	3	3.6 (3, 7)	-2.1 (-2.3, -1.0)
Sentence Assembly	10	NA	NA	NA	NA	NA	3	6.6 (3, 10)	NC
Expressive Language Score†	69	53	57	53	82	50	50	59.1 (50, 82)	-2.7 (-3.3, -1.2)
Total Language Score†	63	60	73	54	80	57	50	62.4 (50, 80)	-2.5 (-3.3, -1.3)
PPVT-R†	65	45	70	NA	62	58	40‡	56.7 (40, 70)	-2.9 (-4.0, -2.0)
TOWK*	6	8	8	NA	9	8	3	7.0 (3, 9)	-1.0 (-2.3, -0.3)

Different subtests were administered at time of assessment, depending on age of child, as indicated by standard CELF-3 test procedures. Each child participated in 6 subtests at each assessment.

NA — not administered; NC — not calculated.

*Normal mean 10, standard deviation 3.

†Normal mean 100, standard deviation 15.

‡Although <40, 40 was used in calculations of summary statistics.

for 6 of the 7 subjects. The mean SDU from the norm mean was -2.0; however, a wide range in achievement was evident, with a minimum of -3.1 and a maximum of -0.5. On the Sentence Structure Subtest, in which the subjects were required to respond to sentences of varying syntactic structures with different levels of complexity, the trend was for below-average performance. One subject achieved a standard score within the average range on this subtest. The subjects demonstrated particular difficulty on the Concepts and Directions Subtest, in which they were asked to follow instructions of increasing length and complexity and which contained a range of language concepts. All results fell below the average range. The subjects' greatest receptive language strength was on the Word Classes Subtest, which assessed their ability to recognize how different word meanings relate to each other (eg, synonyms, antonyms). Five of the 7 subjects achieved standard scores within the average range. The Semantic Relationships Subtest is administered to children 9 years of age and older and assesses the ability to understand semantic relationships within and between sentences. Two subjects participated in this subtest; 1 subject's standard score fell within the average range, and the other's score fell just below the average range.

CELF-3 Expressive Language Score and Subtest Performances. The expressive language scores, derived from a combination of each of the expressive language subtest scores, were well below the average range for 6 of the 7 subjects. Subject 5's score was just below the average range at -1.2 SDUs from the norm mean. The mean SDU from the norm mean was -2.7, but again, a wide range in scores was present, with a minimum of -3.3 and a maximum of -1.2. When expressive morphology was assessed on the Word Structure Subtest, 1 of 5 subjects obtained a result within the average range. The 4 remaining subjects showed below-average skills on this subtest. On the Formulating Sentences Subtest, the subjects' ability to use a specific word or phrase in a sentence to talk about a picture using accurate syntax, semantics, and morphology was an area of consistent difficulty. All subjects showed scores below the average range. When asked to remember and repeat sentences modeled to them on the Recalling Sentences Subtest, 6 of the 7 subjects had extreme difficulty, scoring the lowest standard score possible. One subject achieved a score within the low-average range. The Sentence Assembly Subtest is administered to children from the age of 9 years and assesses the ability to construct sentences from a series of written words

and phrases. Two subjects participated in this subtest; 1 performed within the average range and the other at the lowest standard score possible.

The subjects performed poorly on the PPVT-R, with SDUs ranging from -4.0 to -2.0 and an average of -2.9 . The level of performance of all 6 subjects assessed with the PPVT-R was below the lowest 2.5 percentile expected in a normal-hearing population of a similar age. Expressive vocabulary, as assessed with the Expressive Vocabulary Subtest of the TOWK, was an area of strength for some children; 4 of 6 results were in the average range.

DISCUSSION

After they had used a cochlear implant for 5 years, the 7 subjects had receptive and expressive language skills that were generally below those expected for children of the same age with normal hearing. The intersubject variability in language achievement was consistent with that found by other researchers.^{2,7,8} The subjects also showed significant intrasubject variability across the assessment tasks undertaken. Despite the small sample size, there was some evidence of a pattern of strengths and weaknesses in their language skills.

Details on the rate of language development in the early years after implantation of the subjects in this investigation are not available; however, their overall linguistic skills are not commensurate with those of their hearing peers — a finding that has been reported for some children after implantation.^{4,8} Whether this is due to the eventual slowing of an initially faster-than-normal rate of language development in the subjects, or whether the subjects never reached this pace of development, cannot be concluded. Despite their not reaching skill levels equivalent to those of their age peers with normal hearing, the possibility that the subjects may have maintained a pace of development that has enabled them to stabilize the gap between their language age and their chronological age cannot be ruled out.

Future research may conclude that the amount that the rate of language development increases after implantation might not be sustained in the long term. This finding would reinforce the suggestion that in studies of developmental rate performed so far, children with a cochlear implant have a higher chronological age than their hearing controls (despite having the same language age) and are therefore likely to be at a more advanced cognitive stage. More advanced cognitive development, paired with access to increased sensory input, may explain the observed acceleration in development that takes place immediately after implantation relative to the controls.⁸ It

is not inconceivable that with the passing of time there may be a “dampening” of this effect. This phenomenon may require that predictions about long-term linguistic outcomes compared to those of age-matched hearing peers be considered in more detail.

When considering the language subskills of the subjects, rather than their overall language achievements, we found that the subjects showed a particular strength in using semantic knowledge to recognize how the meanings of words related to each other (eg, synonyms, antonyms). This strength in semantics was also demonstrated in the subjects' expressive vocabulary skills, but was not evident in their receptive vocabulary, which was well below average for all subjects. Given that one would expect a strong relationship between receptive and expressive vocabulary, this effect might be related to factors within the assessment tools, rather than issues specific to the subjects. Further investigation of this relationship is warranted. These findings of relative strengths in semantic knowledge compared to other language skills are consistent with observations of other researchers.^{19,20}

Also consistent with the findings of other researchers was the fact that the subjects' expressive syntactic and morphological development was an area of weakness, compared to their semantic knowledge.¹⁹ This was evidenced by their performance on the Word Structure Subtest and the Formulating Sentences Subtest. Observations of the subjects' errors on the Formulated Sentences Subtest revealed noteworthy difficulties in using accurate morphology and phrase-level syntax, but relatively stronger skills at the clause level. Interestingly, this finding is consistent with observations of children with a profound hearing impairment who use hearing aids,³³ indicating that although children with a cochlear implant may have an overall linguistic advantage, they may still face types of language difficulties similar to those faced by children who use hearing aids.

Consistent weaknesses were present on tasks in which the subjects needed to remember and repeat sentences of increasing length and complexity (Recalling Sentences Subtest) and follow instructions of gradually increasing length and detail involving a range of language concepts (Concepts and Directions Subtest). Both of these tasks require the ability to effectively integrate numerous linguistic and auditory skills. The Recalling Sentences Subtest required use of short-term auditory memory and the linguistic skills of semantics, syntax, and morphology. On the Concepts and Directions Subtest, again, short-term auditory memory is used, as well as language concepts such as sequencing, time, location, and co-

ordination. However, without detailed analysis of the qualitative features of the subjects' responses and their individual approaches to the task, it is difficult to ascertain where their performance broke down and what factor, or combination of factors, contributed to the breakdown.

Variability in achievements across subtests within the group also were found when the skills of each subject were considered individually. This intrasubject variability suggests that individual language subskills were not progressing at equal rates. This pattern, found even at a significant length of time after implantation, supports the assertion that the subjects do not have a language delay from which they may catch up in time. The presence of a language impairment with specific patterns must be considered.

Although SLI is not given as a diagnosis when a child has a significant sensory deficit,³⁴ the similarities in the skill patterns of children with SLI and the subjects with cochlear implants in this study should be considered. In a child with SLI, although the individual language subskills may resemble those of a younger child, the overall profile of his or her language, when the relationships between these subskills are considered, is not typical of normally developing children of a similar age.³⁵ Children with SLI have been noted to show strengths in pragmatic skills and semantic abilities and relative weaknesses in syntax and morphology.²² Although pragmatic skills were not evaluated in this study, the SLI language profile is similar to that of subjects in the study group. Children with SLI have also been noted to generally show average skills on nonverbal IQ tests (despite showing deficits on some nonverbal tasks).³⁵ Again, this finding is consistent with the performance of the subjects in this study. The large discrepancies between verbal IQ and nonverbal IQ scores for 6 of the 7 subjects were of particular concern, implying that the subjects may not be reaching their linguistic potential when considered in light of their other cognitive skills. Finally, in comparison to children with delayed language development, children with SLI may have long-standing language difficulties that change but do not completely resolve over time.³⁵ Again, this observation is consistent with the fact that the subjects in this study have not caught up with their hearing peers after using a cochlear implant for a substantial time. Acknowledging these issues is particularly important because of their implications for developing optimal habilitation programs, implementing school curricula, and understanding possible long-term communication outcomes.

The small sample size and the number of subject variables present in this study prevent us from gener-

alizing these results to the broader population of children with cochlear implants. Even within the sample, explanations of the variables affecting the subjects' performance levels cannot be readily made. For example, subject 5 shows the most consistent performance across subtests, but does not differ greatly on variables of speech perception, communication mode, education, or age at onset of hearing loss from subject 3, who shows average language comprehension skills (Receptive Language Standard Score of 92) but expressive language skills that are well below average (Expressive Language Standard Score of 57).

To assist our understanding of language development after cochlear implantation, the findings of this study need further exploration. First, the relative strengths and weaknesses across different language subskills in a greater number of children need to be studied at regular intervals over an extended period after implantation. This plan will provide more adequate information on the nature of the children's language skills and profile and the course of their development. By specifically looking at the similarities and differences between hearing-impaired children with and without a cochlear implant and children with SLI, we may also come to a greater understanding of the underlying factors that lead to the patterns of performance observed. Second, as suggested by the emerging literature, learning abilities, learning styles, and information-processing strategies and skills must be considered as internal subject variables that could inform us on many of the unexplained factors affecting the performance of both individuals and specific groups of children with cochlear implants.^{25,36,37} Thus, there is a need to analyze the range of interacting processing strategies and auditory and linguistic subskills that affect each other, not only on language assessments, but also in the complexity of linguistic tasks in everyday life that influence a child's day-to-day communication success. Knowledge of these factors will help us make better predictions about a child's likely performance with an implant, evaluate his or her progress more effectively, understand individual performances from task to task more accurately, and intervene in a more informed way to support a child who is showing limited progress. This information may also assist in both understanding and progressing toward reducing the differences in nonverbal IQ and verbal IQ scores.

Finally, research must continue to focus on the needs of our clients with the ultimate aim of enhancing patient outcomes. This goal can be facilitated by discovering what our patients, their families, and associated professionals consider important to derive from future studies. In this way, research findings

can be more directly applied to positive outcomes relevant to the children and families we work with.

In conclusion, this study has shown that 5 years after implantation, each of the children studied showed significant language deficits in 1 or more areas assessed. Despite their having some language skills nearing those of children of the same age with normal hearing, other skills demonstrated, and are

likely to continue to show, severe levels of impairment. Rather than being a pattern of language delay, which implies the chance of catching up in the future, this presentation of language skills is more similar to that of a child with an SLI that may change but not necessarily resolve with the passing of time. The implications of this finding for supporting the development of communicative competence in children with a cochlear implant must be explored in detail.

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COMPARISON OF VARIOUS METHODS OF ELECTROMYOGRAPHIC MONITORING OF THE RECURRENT LARYNGEAL NERVE IN THYROID SURGERY

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In the present study, 3 types of electrodes for recurrent laryngeal nerve (RLN) monitoring are compared: 1) intralaryngeal surface electrodes attached to a conventional endotracheal tube, 2) monopolar needle electrodes placed on the vocal cords by direct laryngoscopy, and 3) bipolar needle electrodes inserted intraoperatively through the cricothyroid ligament. Data were collected from stimulation of 21 RLNs in 16 patients undergoing thyroid surgery. The reliability in terms of distinct electromyographic (EMG) potentials following stimulation of the RLN was 100% with monopolar and bipolar needle electrodes and 76% with intralaryngeal surface electrodes. The mean ($\pm$ SD) amplitudes of the EMG potentials were 1.61 ± 1.6 mV, 2.37 ± 1.8 mV, and 0.35 ± 0.4 mV for the monopolar endolaryngeal, bipolar transligament, and intralaryngeal surface electrodes, respectively. The advantages and disadvantages of each type of electrode are discussed.

KEY WORDS — electromyogram of vocal muscle, recurrent laryngeal nerve monitoring, thyroid surgery.

INTRODUCTION

Recurrent laryngeal nerve (RLN) injury is a feared complication of thyroid surgery. Definitive intraoperative identification of the RLN is essential for reliable preservation of nerve function. As early as 1938, Lahey¹ reported a routine exposure of the RLN in thyroid surgery. Mountain et al² documented a significant decrease of RLN palsy with intraoperative visualization of the nerve. They found that the incidence of nerve paralysis was fourfold greater in cases in which the RLN was not exposed than in cases in which it was exposed.

Various methods have been used to facilitate the identification of the RLN. In all methods, the RLN is electrically stimulated during the operation. However, there are different forms of registration of the elicited laryngeal muscle activity. The simplest is the palpation of the ipsilateral arytenoid cartilage and posterior cricoarytenoid muscle.^{3,4} Spahn et al⁵ modified the procedure by insertion of a needle into the vocal muscle through the cricothyroid membrane and observed the movements of the needle after electrical stimulation of the RLN. Manometric methods used specially designed endotracheal tubes with double cuffs.^{6,7} The lower cuff blocked the trachea, and the upper cuff was placed at the level of the vocal cords. The pressure changes of the upper cuff were registered. However, these methods have been abandoned in favor of electromyographic (EMG) registration of nerve response because of greater reliability.

The EMG methods may be divided into 2 groups that register the EMG potentials either with surface electrodes or needle electrodes. Rea⁸ used a postcricoid surface electrode. Intralaryngeal surface electrodes are integrated in the endotracheal tube or attached to it.^{9,10} However, a comparative study of the systems showed higher EMG amplitudes and less artifact for the intralaryngeal surface electrodes.¹¹ Needle electrodes are used in various modes. Concentric bipolar needle electrodes are inserted intraoperatively through the cricothyroid membrane into the vocal muscle.^{12,13} For monopolar endolaryngeal EMG registration, 2 standard 12-mm subdermal electrodes are placed into the vocal cord via direct laryngoscopy.¹⁴ Other authors have used 2 fine hooked wire electrodes that were placed endoscopically into the vocal cord with a special insertion device.^{15,16}

The purpose of the present study was to compare the intralaryngeal surface electrode, the bipolar transligament electrode, and the monopolar endolaryngeal needle electrodes in a prospective clinical evaluation of electrophysiological monitoring of the RLN during thyroid surgery.

MATERIAL AND METHODS

From January to July 2001, 16 consecutive patients undergoing thyroid surgery were enrolled in the study. The underlying diseases were benign multinodular goiter ($n = 13$), papillary carcinoma ($n = 2$), and toxic adenoma ($n = 1$). Five patients underwent bilateral operation. Thus, 21 RLNs were at risk and could be

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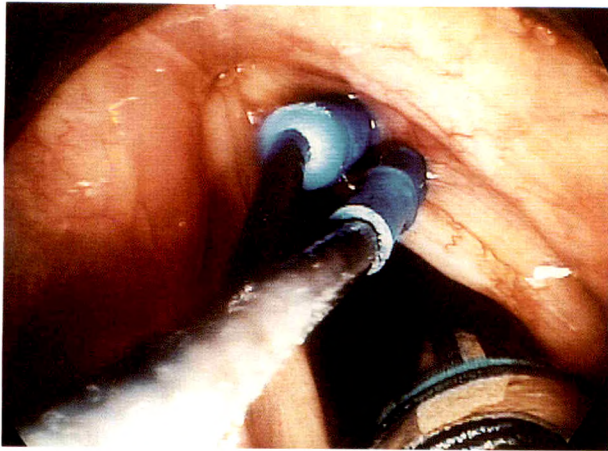


Fig 1. Surface electrode and monopolar electrodes seen in direct laryngoscopy.

monitored. In 1 patient, there was a preoperative RLN palsy due to poliomyelitis that had been incurred 40 years earlier.

In a prospective clinical study, the intralaryngeal surface electrode, the monopolar endolaryngeal needle electrodes, and the bipolar transligament electrode were placed in all patients. A standard EMG machine (Neurosign 100) with an integrated nerve stimulator was used. Additionally, EMG signals were depicted by online documentation software (Inomed, Teningen, Germany) for graphic display and further analysis (waveform and duration of EMG potentials). After surgical exposure, the RLN was stimulated by a concentric bipolar probe. The stimulating current was set at 0.7 mA, and the stimulation frequency was 3 Hz. The electrode impedance was constantly monitored by the Neurosign 100. Thus, any dislocation of the electrode or defective wiring was easily checked.

The intralaryngeal surface electrode (Xomed-Treace) consists of a flexible plastic body. One side of the plastic body features a printed circuit that is in electrical contact with the vocal cords. The other side of the electrode carries an adhesive pad for attachment of the electrode to a conventional endotracheal tube 10 mm above the cuff. Placement of the electrode and intubation was performed by the anesthesiologist.

For monopolar endolaryngeal EMG registration, 2 standard 12-mm subdermal electrodes were used (Fig 1). After visualization of the larynx by direct laryngoscopy, the electrodes were grasped by a forceps and set into the vocalis muscle and lateral cricoarytenoid muscle. Care was taken not to dislocate the electrodes during the withdrawal of the laryngoscope. The time taken for the placement procedure was registered.

The bipolar transligament electrode was placed in-

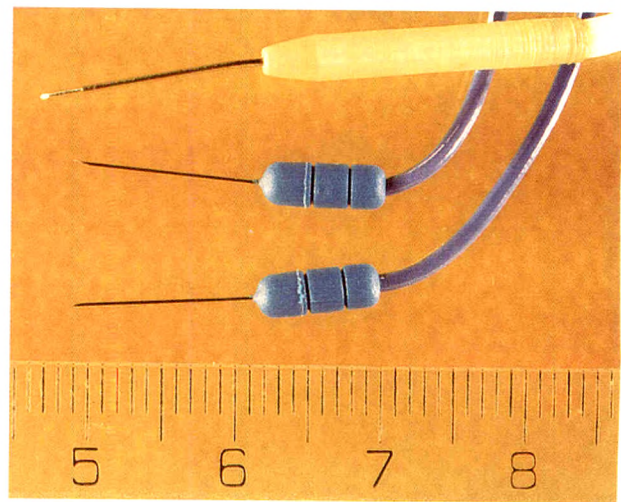


Fig 2. Tips of bipolar and monopolar needle electrodes. Rule is in centimeters.

traoperatively after exposure of the prelaryngeal region. The electrode was introduced into the vocalis muscle and lateral cricoarytenoid muscle via the cricothyroid ligament in a laterocranial direction at an angle of about 30°. Then, the electrode wire was connected to the preamplifier of the Neurosign 100 (Fig 2). The number of punctures taken to obtain EMG potentials was noted.

No restrictions were set concerning the relaxation of the patients during anesthesia. A high degree of relaxation was actually desired, because it is well known that swallowing movements may easily dislocate the monopolar endolaryngeal needle electrodes.¹⁷ These electrodes are straight and lack a hook to prevent extrusion. On stimulation of the RLN, the degree of patient relaxation was assessed by the twitch response of the adductor pollicis muscle following train-of-four stimulation of the ulnar nerve.

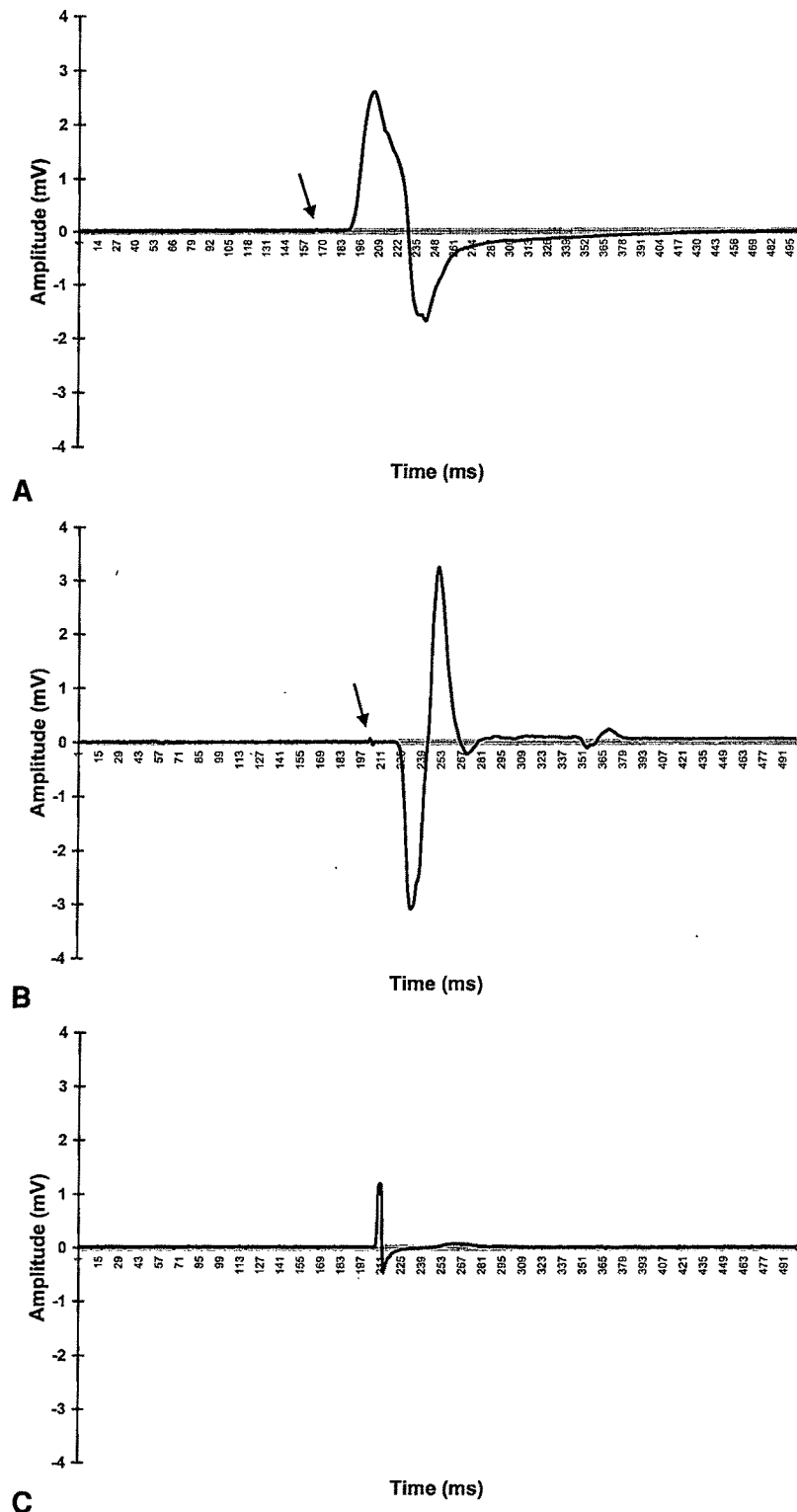
Stimulation of the RLN was in random order for the 3 types of electrode systems. The maximal amplitude of the signals was recorded. Moreover, the waveform and duration of the EMG potentials were analyzed. A Wilcoxon signed-rank test was used for statistical analysis, and the level of significance was set at $p < .05$.

The vocal cords of each patient were checked on the first postoperative day by fiber laryngoscopy.

RESULTS

After stimulation of the RLN, reliable EMG potentials could be registered in all cases with the monopolar endolaryngeal and the bipolar transligament electrodes. With the intralaryngeal surface electrode, reliable EMG potentials were evoked in only 16 of the 21 stimulated RLNs, and no EMG potentials were observed in 5 cases. The degree of muscular relaxa-

Fig 3. Graphic display of typical waveforms of electromyographic potentials obtained with A) endolaryngeal monopolar electrodes, B) bipolar transligament electrode, and C) intralaryngeal surface electrode. Arrows — stimulation artifact.



tion was on average ($\pm$ SD) 0.67 ± 0.7 twitch after train-of-four stimulation of the ulnar nerve. This relatively high degree of muscular relaxation at the time of RLN stimulation may have contributed to the absent EMG responses with the intralaryngeal surface electrode. In 4 cases of missing EMG potentials, the patients had 0 twitch, indicating near-complete mus-

cular relaxation. However, the disposable surface electrode was found to be detached from the endotracheal tube at its withdrawal in 2 patients with 2 stimulated RLNs. No case of displacement of the electrode was indicated by impedance surveillance. The patient with preoperative RLN due to poliomyelitis showed reliable EMG potentials within normal

limits with all 3 types of electrodes.

The maximal amplitudes of EMG potentials were on average ($\pm$ SD) 1.61 ± 1.6 mV, 2.37 ± 1.8 mV, and 0.35 ± 0.4 mV for the monopolar endolaryngeal, bipolar transligament, and intralaryngeal surface electrodes, respectively. A typical waveform for each type of EMG electrode is shown in Fig 3. The mean ($\pm$ SD) durations of the compound action potential were 14.7 ± 2.7 ms, 4.3 ± 0.4 ms, and 1.7 ± 0.3 ms for the monopolar endolaryngeal, bipolar transligament, and intralaryngeal surface electrodes, respectively. All differences of amplitude and duration of the EMG potentials were significant for the 3 types of electrodes.

The time taken for direct laryngoscopy and placement of the monopolar endolaryngeal electrodes was on average ($\pm$ SD) 8.1 ± 3.1 minutes. Direct laryngoscopy was performed without problems in all patients.

Reliable EMG potentials were obtained with the bipolar transligament electrode in 17 of the 21 cases at the first puncture and in 4 of the 21 cases at the second puncture. No more than 2 punctures were necessary in any case. The vocal cords had a normal aspect on the first postoperative day in all 4 patients with 2 punctures. Thus, an additional puncture was not associated with an increased incidence of hematoma of the vocal cords. In 1 patient, placement of the bipolar transligament electrode was hampered by a gross nodular goiter with a weight of 450 g. The electrode could only be placed after extensive mobilization of the upper lobe of the goiter, which was a prerequisite for sufficient exposure of the cricothyroid membrane.

Fiber laryngoscopy on the first postoperative day showed normal vocal cord function except for the 1 patient with preexisting palsy of the RLN. A slight hematoma of the vocal cord was found in 3 cases and resolved spontaneously within a few days.

DISCUSSION

The largest amplitude EMG potentials were found with the bipolar transligament electrode, followed by the endolaryngeal monopolar electrodes, with mean amplitudes of 2.37 mV and 1.61 mV, respectively. With these EMG potentials, the Neurosign 100 produced loud acoustic signals of low pitch that could easily be distinguished from stimulation artifacts. However, the EMG potentials with the intralaryngeal surface electrode were considerably smaller, with an average amplitude of 0.35 mV. It is well known from electrophysiology that surface electrodes produce smaller potentials than needle electrodes for reasons of electrical impedance. The mucus of the tracheal

secretions may additionally impair the transmission of electrical signals between the vocal cords and the surface electrode.

The EMG potentials below 0.2 mV may be acoustically confused with direct stimulation artifacts. The acoustic signals from true EMG potentials and stimulation artifacts are of low pitch and high pitch, respectively. Precise distinction is possible by a graphic display showing a typical biphasic EMG potential following stimulation of nerve and a monophasic potential in the case of stimulation artifacts. However, the Neurosign 100 is not routinely fitted with a graphic display.

The bipolar transligament electrode and the endolaryngeal monopolar electrodes showed consistently reliable EMG responses in 100% of the stimulated RLNs — even in patients with near-complete muscular relaxation. However, only 76% (16/21) had reliable EMG potentials with the intralaryngeal surface electrode. Using the same surface electrode, Gericke and Müller¹⁷ and Horn and Röttscher¹⁸ found reliabilities of 80% and 93%, respectively. In both studies, only short-acting depolarizing agents were allowed for the induction of anesthesia. The lower rate of reliability in our study may be partly explained by the fact that no restrictions were made concerning muscular relaxation. However, in 2 cases, the surface electrode was found to be detached from the endotracheal tube. Our data suggest a higher reliability with needle electrodes (bipolar transligament and monopolar endolaryngeal electrodes) than with the intralaryngeal surface electrode.

The analysis of the waveforms of the EMG potentials revealed distinct patterns of monopolar and bipolar needle electrodes. The potentials from the endolaryngeal monopolar electrodes were longest, with an average duration of 14.7 ms. The long duration may be explained by the position of the monopolar electrodes, which are generally placed about 6 to 8 mm apart. With the bipolar transligament electrode, potentials were significantly shorter, with a mean duration of 4.3 ms, because of the extremely short distance between the concentric electrodes located at the point of the needle. The very short duration of EMG potentials seen with the intralaryngeal surface electrode, with a mean of only 1.7 ms, may be attributed to the small amplitude and the different behavior of surface electrodes as compared to needle electrodes.

Hooked wire electrodes consist of fine platinum wires that are inserted into the vocal cords with a special adapter.^{15,16} In the present study, hooked wire electrodes were not used for several reasons: 1) the electrical impedance of hooked wire electrodes is

PROPERTIES OF ELECTRODES USED FOR INTRAOPERATIVE MONITORING OF RECURRENT LARYNGEAL NERVE

	<i>Bipolar Transligament Electrode</i>	<i>Monopolar Endolaryngeal Electrodes</i>	<i>Intralaryngeal Surface Electrode</i>
Mean ($\pm$ SD) amplitude of electromyographic potentials (mV)	2.37 $\pm$ 1.8	1.61 $\pm$ 1.6	0.35 $\pm$ 0.4
Reliability (%)	100	100	76
Muscle relaxation during stimulation	Permitted	Permitted	Not permitted
Reusability of electrode	Reusable	Reusable	Single-use
Disadvantages	Insertion difficult in large goiters; electrode wire may obstruct operative field	Direct laryngoscopy necessary; electrode placement by skilled otolaryngological surgeon; mean insertion time: 8 minutes	Small electromyographic potentials may be confused with stimulation artifacts

fivefold greater than that of conventional electrodes; 2) the handling of the extremely fine platinum wires (0.002 inch in diameter) is cumbersome; and 3) the electrodes are costly and for single use only.

Practical considerations are also important in making the decision as to which electrode is most suitable for a given clinical situation. Anesthesia is facilitated if no restrictions are made concerning muscular relaxation, as is possible when the bipolar transligament electrode and the endolaryngeal monopolar electrodes are used. Direct stimulation of the surgically exposed RLN consistently allows the registration of reliable EMG potentials even if the anesthesiologist finds no muscular contraction after routine transcutaneous stimulation of the ulnar nerve. However, only short-acting depolarizing agents should be allowed with the intralaryngeal surface electrode. Direct laryngoscopy is necessary for the placement of endolaryngeal monopolar electrodes. In general, the skills of an experienced otolaryngological surgeon are required for easy performance. However, direct laryngoscopy may not be possible for anatomic reasons of the neck, as, for instance, in Bechterew's disease. On the other hand, the intralaryngeal surface elec-

trode may be positioned by the anesthesiologist. Insertion of the bipolar transligament electrode can be difficult in large goiters with a redundant superior lobe and isthmus. Moreover, the electrode wire may disturb the operating field. Advantages and disadvantages of the different types of electrodes are summarized in the Table.

The present study confirms a high reliability and practicability of the endolaryngeal monopolar electrodes and the bipolar transligament electrode for RLN monitoring in thyroid surgery. Both types of electrodes are reusable and therefore cost-effective. Needle electrodes should be preferred over surface electrodes for electrophysiological reasons whenever possible. The intralaryngeal surface electrode may be indicated, however, if 1) direct laryngoscopy is not available or is impossible for anatomic reasons and endolaryngeal monopolar electrodes cannot be placed or 2) insertion of the bipolar transligament electrode is difficult because of an extremely large goiter. Precise knowledge of the advantages and disadvantages enables the clinician to make the appropriate choice of the electrode type for RLN monitoring in thyroid surgery.

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SPONTANEOUS INTRACRANIAL HYPOTENSION: A RARE CAUSE OF LABYRINTHINE HYDROPS

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Spontaneous intracranial hypotension should be considered as a possible cause of cochlear hydrops. We report a case of unilateral hearing loss attributed to spontaneous intracranial hypotension on the basis of characteristic abnormalities seen on magnetic resonance imaging. The diagnostic gold standards for intracranial hypotension are lumbar measurement of cerebrospinal fluid pressure and magnetic resonance imaging. The usual treatment is an autologous blood injection into the peridural spaces. The mechanism of hearing loss is thought to involve secondary perilymph depression due to a patent cochlear aqueduct. This perilymph depression would induce a compensatory expansion of the endolymphatic compartment, with a subsequent decrease in basilar or Reissner's membrane compliance. Endolymphatic hydrops can occur in the course of intracranial hypotension, and not only because of abnormal endolymph production or resorption. Hydrops can thus be classified into 1) syndromes of endolymphatic origin and 2) syndromes of perilymphatic origin, in which loss of perilymph induces compensatory expansion of the endolymphatic space.

KEY WORDS — cerebrospinal fluid, cochlear aqueduct, headache, hearing loss, hydrops, spontaneous intracranial hypotension.

INTRODUCTION

Spontaneous intracranial hypotension (SIH) is a rare condition first described in 1938 by Schaltenbrand.¹ The advent of magnetic resonance imaging (MRI) renewed interest in SIH.

The most prominent feature of this syndrome is profound orthostatic headache provoked by sitting or standing and relieved by lying flat. Associated symptoms may include postural tinnitus, vertigo, nausea, and mild neck stiffness.²

We report a case of unilateral hearing loss attributed to SIH on the basis of characteristic MRI abnormalities. Specific treatment yielded complete clinical recovery and MRI normalization. Spontaneous intracranial hypotension should be considered as a possible cause of cochlear hydrops.

CASE REPORT

A 37-year-old man gradually developed severe, generalized headache. It was postural, occurring in the upright posture and being relieved within a few minutes of lying flat. Associated features included "echoing" in his ears, mild dizziness, and hearing loss on the left side. He was unable to work and was admitted 3 weeks after onset. At entry, he was apyrexial and well hydrated, with unremarkable neurologic findings. The findings on otoscopy were normal, and pure tone audiometry showed mild unilat-

eral hearing loss on the left side, affecting the lower frequencies. The stapedial reflex was preserved. Interestingly, the hearing loss seemed to fluctuate with changes in body position. In particular, lying flat improved the hearing of low frequencies on 3 consecutive audiograms (average curve in Fig 1A). Cerebral MRI revealed 2 characteristic signs: 1) diffuse bilateral subdural collections on T2-weighted acquisitions (Fig 2A); and 2) meningeal enhancement on T1-weighted acquisitions following administration of gadolinium (Fig 2B).

A lumbar blood patch improved the symptoms (headache, resonant distortion, tinnitus, and deafness; Fig 1B) and led to the disappearance of the dural enhancement (Fig 2C). The patient remains without symptoms 12 months later.

DISCUSSION

Mechanisms of Spontaneous Intracranial Hypotension. Spontaneous intracranial hypotension is considered rare^{2,3} relative to low-pressure headaches following lumbar puncture, myelography, and spinal surgery. Spontaneous intracranial hypotension is defined by a cerebrospinal fluid (CSF) pressure of less than 60 mm H₂O in a patient without a recent history of lumbar puncture or neurosurgery.⁴ Reduced production, increased resorption, and leakage of CSF are all possible etiologic mechanisms. Several arguments, however, support the leakage hypothesis.

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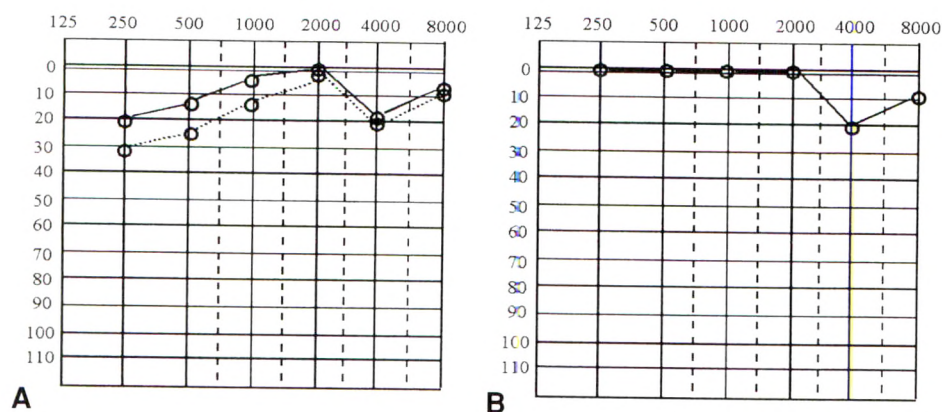


Fig 1. Left pure tone audiograms. **A)** At entry, showing lower-frequency hearing loss with postural changes. Dotted line — in upright posture; continuous line — after few minutes of lying flat. **B)** After lumbar blood patch, showing significant improvement in hearing of lower frequencies associated with clinical recovery.

First, spontaneous CSF leakage has been shown in the cervical, dorsal, or lumbar spine by radionuclide cisternography,⁵ contrast myelography,^{2,6} and spinal MRI.⁷ Second, a history of minor head injury or vigorous exercise is frequent in SIH,⁸ possibly resulting in dural tears on vulnerable lumbar roots.

Cranial nerve impairment has been reported in the course of SIH (sixth nerve paralysis⁹). However, postural headache (which appears in the upright position and improves in the supine position) remains the cardinal sign of SIH.¹⁰ Postulated mechanisms include sagging of the brain, dilation of intracranial veins, traction of pain-sensitive tethering veins (which suspend the brain from the large dural sinuses), and activation of adenosine receptors.¹¹ Observed subdural hematoma, however, supports the hypothesis of traction of tethering veins.

Mechanism of Hearing Impairment. It is well known that intracranial hypotension can affect hearing thresholds — for example, as after spinal anesthesia (3% of reversible hearing loss¹²) and intracranial surgery (20% of contralateral hearing loss after

acoustic neuroma surgery¹³). The severity of hearing loss and the number of frequencies involved correlate with the amount of CSF lost.¹⁴

Under normal conditions, the pressures in the CSF, perilymph, and endolymph are equal.¹⁵ However, the cochlear aqueduct may remain patent in some individuals, so that CSF pressure variations might be transmitted more quickly to the perilymph compartment than to the endolymph compartment. Indeed, the fact that the endolymphatic sac is enclosed in the dura may lessen the pressure transmission. In SIH, CSF loss may secondarily induce perilymph depression with compensatory expansion of the endolymphatic compartment¹⁶ and a subsequent endolymphatic hydrops.^{17,18} Thus, endolymphatic hydrops might occur not only because of abnormal endolymph production or resorption,¹⁹⁻²¹ but also in the course of intracranial hypotension. If so, hydrops may be classified into 1) syndromes of endolymphatic origin (Meniere's disease, autoimmune processes) and 2) syndromes of perilymphatic origin, in which loss of perilymph induces compensatory expansion of the

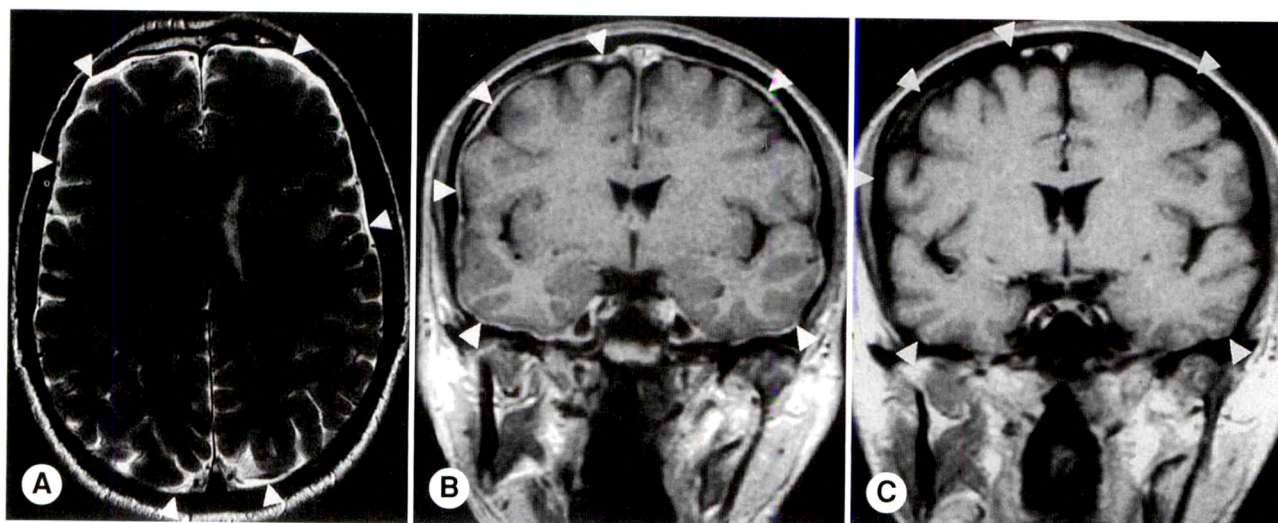


Fig 2. Magnetic resonance images. **A)** T2-weighted axial image shows bilateral subdural collections (arrowheads). **B)** T1-weighted coronal image with gadolinium contrast shows extensive meningeal enhancement (arrowheads). **C)** Disappearance of meningeal enhancement 2 weeks after lumbar blood patch on T1-weighted images with gadolinium (arrowheads).

endolymphatic space. The latter may be observed in a variety of conditions, such as perilymphatic fistula (spontaneous or traumatic) and intracranial hypotension with a patent cochlear aqueduct.

It is unclear why hearing loss is so rare in the course of SIH. It may be absent in the unknown proportion of patients with a lack of patency of the cochlear aqueduct.²² In addition, some patients are probably misdiagnosed because of other major clinical complaints (eg, headache, oculomotor nerve paralysis) or because only a few frequencies are involved.

Diagnosis and Treatment. The diagnostic gold standard for intracranial hypotension is direct measurement of CSF pressure by lumbar puncture, but noninvasive techniques have been developed. The tympanic membrane displacement measurement technique has been used to provide reliable measures of perilymphatic pressure and CSF-perilymphatic pressure transfer.²³ Magnetic resonance imaging shows characteristic signs of intracranial hypotension, namely, 1) abnormal, intense, diffuse, symmetric, and contiguous enhancement of the supratentorial and infratentorial dura, including the convexities, interhemispheric fissure, tentorium, and falx, and 2) abnormal dural venous sinus enhancement suggesting compensatory venous expansion.²⁴ These aspects (subdural collections and dural enhancement) are observed as a result of the decreased CSF volume in the course of post-lumbar puncture headache, as well as in the SIH syndrome.

Use of color Doppler flow imaging to measure blood flow in the superior ophthalmic vein has been successfully used for the diagnosis of intracranial hypotension,²⁵ but it remains underused in the diagnosis of SIH. Cisternography and myelography can be used

to locate the site of leakage or excessive resorption, but these examinations are not generally required; in a patient with typical clinical and imaging signs, neither CSF pressure measurement nor precise location of the leakage point is required for the diagnosis.

Empirical treatment includes bed rest and administration of caffeine, corticosteroids, or mineralocorticoids.⁴ However, the most widely used treatment, based on the leakage hypothesis, is the epidural blood patch. This technique consists of injecting autologous blood into the peridural spaces so as to form a hematoma. Thirty milliliters of blood is injected — less, if the patient feels dorsal pain. It is not necessary to know the precise location of the leak, as the blood clot distributes itself over approximately 10 medullary segments. This technique was successfully used in our patient.

CONCLUSION

Spontaneous intracranial hypotension is a rare syndrome defined by postural headache associated with low CSF pressure (<60 mm H₂O) in the absence of dural puncture or surgery. Vertigo, tinnitus, and “echoing” in the ears are frequently part of the SIH syndrome, but hearing loss with a postural component is rare. The case we present suggests that labyrinthine signs in the course of SIH are related to hydrops of perilymphatic origin. This observation would allow hydrops to be classified as the consequence of either 1) endolymphatic hypertension (Meniere’s disease, autoimmune processes) or 2) perilymphatic depression with compensatory expansion of the endolymphatic space (perilymphatic fistula, or intracranial hypotension, in the case of a patent cochlear aqueduct).

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NONCONTACT THREE-DIMENSIONAL LASER MEASURING DEVICE FOR TRACHEOSCOPY

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Luminal dimension measurements are still an unsolved problem in endoscopy. The goal of our study was to develop a 3-dimensional (3-D) measuring device for endoscopic applications. For this purpose, a fiber probe projecting a ring of laser light (laser diode, 675 nm) was integrated in a position registration system. Software was developed for image analysis, distortion correction, and export of data for 3-D display. Experimental evaluation of measuring accuracy employing plastic tubes and 15 postmortem pig trachea preparations with artificial stenosis indicated a high level of method precision (Pearson's correlation coefficients, $r = 0.99$ for normal tracheal lumen and $r = 0.97$ for high-grade stenosis; $p < .0001$). Our clinical experience with the technique in 10 patients with tracheal stenosis revealed no side effects and encourages us to recommend this method for other endoscopic applications. The method enhances our 3-D grasp of endoscopically examined lesions.

KEY WORDS — airway, diagnostics, endoscopy, laser, measuring methods, three-dimensional imaging, tracheal stenosis.

INTRODUCTION

Diagnostic and therapeutic use of endoscopes is still on the increase. Excellent endoscopic image quality and documentation are now obtainable. It must be kept in mind, however, that these images do not represent the true size of endoscopically examined human body structures. The problem is inherent to the optical characteristics of endoscopes. The telescoping effect of the endoscope lenses causes stenosis located at a distance from the tip of the endoscope to appear higher-grade, and stenosis detected very close to the endoscope tip to appear lower-grade, than is actually the case. The miniature optics of current endoscopes have a second negative aspect: the images are spherically distorted. The size, quality, and color of the endoscopic images are also influenced by the settings of the charge-coupled device (CCD) cameras, picture amplifiers, and monitors used.

Endoscopic findings must therefore be critically evaluated by the examiner. The endoscopy report unavoidably reflects personal experience and subjective estimations, rendering objective comparison of such reports difficult, especially when different examiners have been at work.

Based on these considerations, our goal was to develop a new measuring device for tracheoscopy that would enable physicians to compare findings, to measure diameter, cross-sectional area, and stenosis

length, and to obtain a 3-dimensional (3-D) view of the airway. The need for such a tool is felt keenly in cases of benign and malignant stenosis of the aerodigestive tract and may also prove useful for pharyngeal obstruction during snoring, stenoses of the blood vessels, urogenital tract, and bile duct, measuring expansion of tumors and ulcers in the gastrointestinal tract, and numerous other medical applications.

METHOD

New Device and Our Technique. Our method combines laser-ring marking of a discrete cross section of the trachea with a probe position sensor integrated in a true-image 3-D tracheoscopic measuring system. Laser light delivered from a laser diode (LQ6-675, HIV GmbH, Wadgassen, Germany; wavelength, 675 nm) is bundled and sent through a single-mode laser fiber. This marking laser fiber can be introduced as a probe into the suction channel of a flexible endoscope or is guided adjacent to the housing of a rigid endoscope.

The distal end of the laser fiber is connected to a device that reflects the laser light delivered by the fiber optics in a transverse, circular plane so that a ring of red light is projected onto the internal surface of the tracheal wall (Fig 1). The size and form of this laser ring incorporates the desired information on the diameter and cross-sectional area of the marked lumen segment.

In tracheoscopy, the laser marking fiber is adjusted

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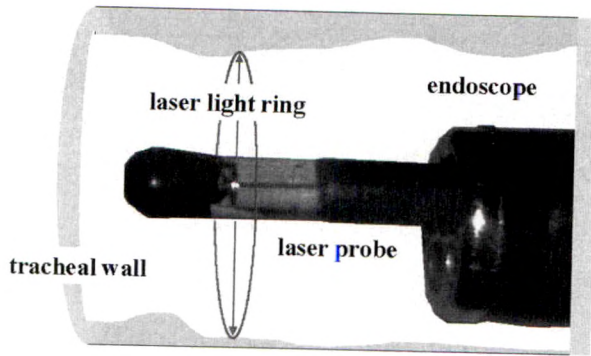


Fig 1. Laser fiber tip. Laser fiber probe is inserted into suction channel of flexible endoscope. Circular reflecting device at tip of probe generates ring of red laser light onto inner surface of tracheal wall.

by the endoscopic examiner so that the axis of the fiber corresponds to the axis of the trachea in the flexible or rigid bronchoscope. This step ensures that the actual cross section of the trachea is measured. The maximum margin of measuring error due to a nonparaxial positioning of the laser fiber in relation to the tracheal axis is $<1\%$.

The relative magnification of the laser ring can be determined mathematically on the basis of the known distance between the ring and the distal end of the endoscope. The image spatial distortion error resulting from the spherical aberration of the integrated microlens optics was corrected by standardizing the measuring system for individual endoscopes.

When a beam splitter is added to the eyepiece of the endoscope, one copy of the endoscopic image information is made available for image processing, and the other can be examined by the endoscopist. A bandpass filter was coupled to a CCD camera (C-CS8320BC, NET GmbH, Gerlinden, Germany). Wavelengths in the area of the laser spectrum were filtered out of the illuminating white light to keep it from whitening out the measuring image.

The outer surface of the laser probe was marked with incremental black rings (Fig 2). We developed

a noncontact sensor to read the ring pattern. The sensor records the movement of the laser probe in 1-mm steps, and the z-coordinates required for 3-D measurement and display are determined and stored.

The image processing was done on a standard IBM-compatible personal computer by means of software also developed by our team (Endoscan 3.0). The software provides for capture and analysis of images in 1-mm steps during tracheal endoscopy (Fig 3). After image processing, distortion correction, and pixel-to-millimeter correction, the detected contour of the laser ring was stored in a data table comprising 360 points for each segment. We used commercial 3-D viewer software (3D View 3.5, Actify Inc, San Francisco, California) to render the measurement results 3-D.

Plastic Cylinder Model. In preliminary testing, we used transparent acrylic glass cylinders with drilled holes of different diameters (20, 18, 16, 15, 14, 12, 11, and 10 mm) and marks on the surface at defined intervals (6, 12, 18, 24, 30, 36, 42, and 55 mm). The tubes were examined 15 times by our measuring method. The cross sections and distance values were compared with the known dimensions of the acrylic tubes.

Pig Tracheal Model. Fifteen fresh postmortem pig tracheal preparations (A, B, C, D, E, F, G, H, K, L, N, O, Bl, Xy1, and Xy2) were used to estimate the precision of our measuring method under clinically relevant conditions. Various stenotic forms were induced in the pig trachea. Seven pig tracheas were deformed by means of external compression in a semifrozen state, resulting in 10% to 70% tracheal stenoses of the malacia type. We created 3 ring stenoses by means of submucous placement of a loop of surgical suture material. Two laryngotracheal junctions and 2 unchanged tracheal segments of normal luminal dimensions were also examined as reference material. Finally, we simulated a tracheotomy-like lesion in 1 case. After marking the starting point for later measurement with a pin, we fixed the trachea

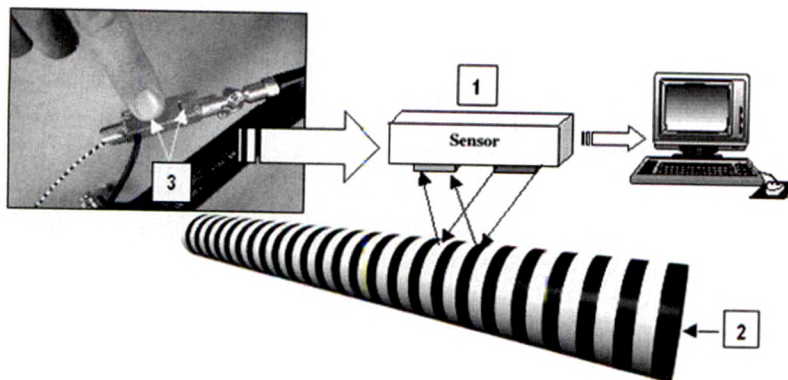


Fig 2. Laser probe motion detector (1). Two reflection light barriers count number of black stripes on laser probe coating that have passed sensor. Two key switches (3) are used as follows: key 1 resets entire system to 0, and key 2 is used to mark beginning of pathological finding. On basis of known stripe width and distance between two stripes (2), actual position of probe can be determined and stored on computer.

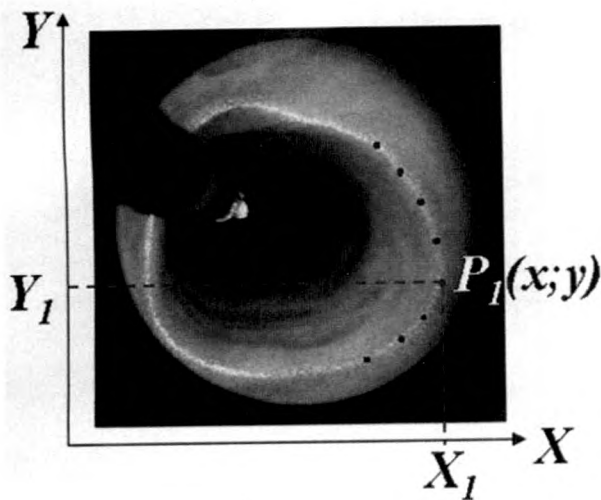


Fig 3. Segmentation of gray scale image. In 1° steps from center of image, special edge detection software calculates x- and y-pixel positions of "white" laser ring.

form with a plaster coat and froze the entire tracheal preparation. Endoscopic measurement of the preparations was done with a rigid endoscope (0° Hopkins Optic, Karl Storz, Tuttlingen, Germany) in a fixed mount that was moved in 1-mm increments beginning at the needle-marked starting point. The cross-sectional plane of measurement was thus advanced from image to image. The total length of the segment measured in each tracheal preparation was 50 mm.

The precision of the endoscopic measuring technique was checked against silicone copies of the tracheal cavities. The casts were made after endoscopic analysis of the frozen plaster-coated tracheal segments to exclude any changes in the tracheal form. We used Sanoform (Sanomed, Hamburg, Germany) to prepare the silicone copies. After polymerization of the silicone material, the plaster coat and tracheal tissue were removed. The control measuring procedure was carried out with a highly precise Cylan 3-D laser scanner (Intecu GmbH, Gera, Germany) with a deviation range of less than $30\text{ }\mu\text{m}$. Six of the reference silicone copies (B, L, N, Bl, Xy1, and Xy2) were excluded from the comparison procedure because of rotation instability of the silicone body during measuring. We tested the precision of our method of measurement by means of sequential comparison of the endoscopic measurement with the cross-sectional areas calculated by the Cylan scanner for each of the 50 "cross sections" per trachea.

Clinical Evaluation. Practical evaluation was done by testing the clinical applicability of the measuring system method in 10 cases of routine upper respiratory tract endoscopy in patients who had declared informed consent to the procedure. In 4 cases, the tracheoscopy was planned as a rigid endoscopy, and

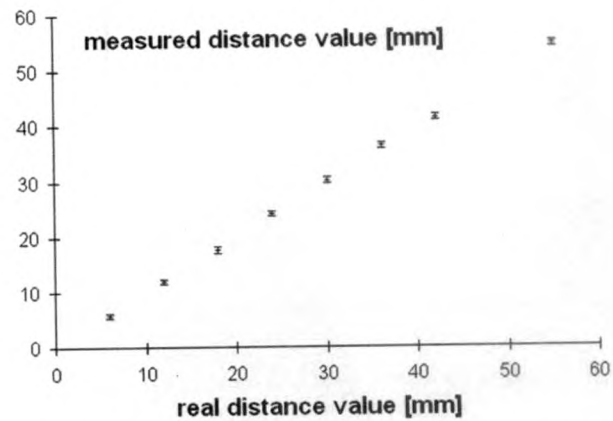


Fig 4. Distance measurement. Comparison of median measured distance values (marked with "+") to known distance values showed strongly linear correlation with minimal standard deviation values (marked with "—"): Pearson's correlation $r = 0.99$; $p < .0001$; $n = 120$.

so the measuring technique was adapted to the rigid endoscope. In the other 6 cases, the tracheoscopy was done with a fiberscope under local anesthesia. All of the patients involved had tracheal stenosis. The 4 rigid endoscopic examinations were performed together with therapeutic interventions (3 stent implantations and 1 laser incision of a scar). The flexible endoscopic procedures were indicated for surveillance purposes after laryngeal and tracheal surgery.

Statistics. Statistical comparison of the true distance and cross-sectional area values and the values obtained with our new measuring device was done with Pearson's correlation coefficient and the corresponding confidence intervals. The graphical visualization of measuring accuracy in relation to reference values and corresponding confidence intervals was obtained by linear regression with the Passing and Bablok plot.¹

RESULTS

Experimental Results. Our preliminary measurements of the acrylic glass cylinders validated our distance and cross-sectional area measuring system for the dimensions prevalent in central airways. The transparent acrylic glass cylinder allowed for highly exact and reproducible positioning of the laser light ring at the respective measuring points marked on its surface.

The distances measured with our new laser probe position sensor (Fig 2) and the cross-sectional area measurement (Fig 3) corresponded closely to the true dimensions of the acrylic cylinder. In 15 completed measuring cycles, the distance values were demonstrated to be readily reproducible. The maximum deviation among measuring values of the set point totaled 1 mm. No progression of measuring error with

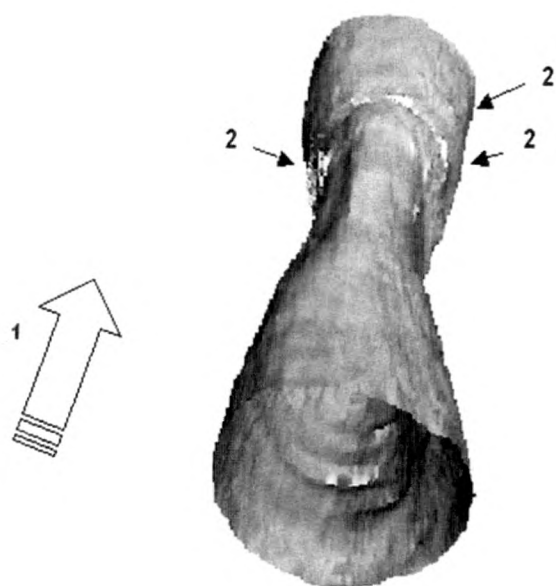


Fig 5. Three-dimensional (3-D) visualization. Three-dimensional reconstruction of endoscopically measured tracheal segment (trachea F) generates good spatial impression of stenosis. White arrow (1) shows measuring direction. Behind stenosis, small irregularities are seen on surface of tracheal wall (black arrows; 2).

increasing measuring length was observed (Fig 4).

Our endoscopic 3-D measuring procedure and the 3-D image reconstruction of the results was successfully completed in all 15 postmortem pig tracheal preparations (Fig 5). Tracheas (A) without artificial stenosis and with low-grade stenosis (C, D) revealed superior measuring accuracy of the Endoscan system with a Pearson's correlation of $r = 0.99$ ($p < .0001$) compared to the reference values supplied by the Cylan laser scanner (trachea A in Fig 6¹). High-grade stenosis as in trachea F was also measured endoscopically and correlated closely with the reference values of the industrial laser scanner (Fig 7¹).

The Passing and Bablok¹ plot shows a slightly increased deviation range for the measuring results on

the cross-sectional area values between 92 mm² and 104 mm². The measured area values of the actual stenotic region (86 to 92 mm²) did not show this divergence. The total of 50 cross sections compared for trachea F showed a good Pearson correlation ($r = 0.97$, $p < .0001$) of endoscopic and reference measuring values.

Clinical Results. We evaluated the clinical efficiency of our Endoscan system in 10 cases of tracheal stenosis. The Table lists the individual indications for tracheoscopy and the subjective evaluation of clinical applicability given by the examiner. The better image quality of rigid endoscope optics made automatic image analysis and laser ring detection easier with rigid scopes than with flexible endoscopy.

Two problems were encountered in a case of laser incision of a tracheal stenosis (patient 3). First, we could not measure the tracheal lumen behind the stenosis correctly, because the technique requires a laser ring to be positioned at a defined distance from the tip of the endoscope. Thus, while the laser probe is still passing through the stenosis, the endoscope itself is not. Therefore, the laser ring comes to lie in the shadow of the stenosis. The second problem was compromised optical quality due to blood and secretions during surgery. Better results were obtained by performing the tracheoscopy several days after surgical intervention under local anesthesia.

Using flexible endoscopy, we were able to reach and measure all of the relevant tracheal segments. Because of the lower image quality with these scopes, we had to assist the image processing. The main advantage of endoscopy in non-narcotized patients is the possibility of analyzing dynamic changes in airway size.

In the cases of patients 5 and 9, the tracheoscopy and the measuring process were problematic because of poor patient compliance, hypersalivation, and motion artifact.

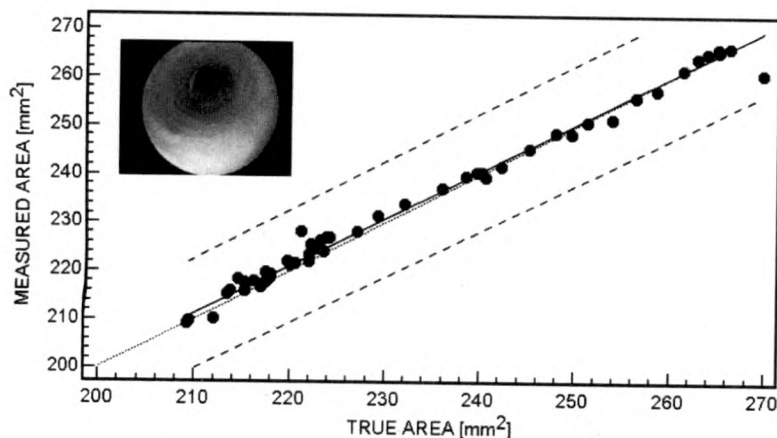
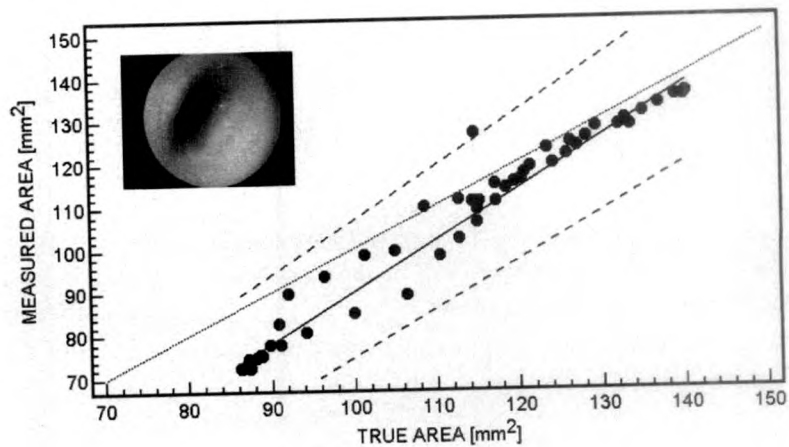


Fig 6. Cross-sectional area measurement in trachea A, without stenosis. Comparison between endoscopically measured cross-sectional area and true cross-sectional area was obtained with Cylan 3-D scanner. Passing and Bablok¹ plot was used to compare new measurement with established one. Dotted lines show linear regression of true values (Cylan scanner). Bullets show endoscopically measured values ($n = 50$). Dashed lines show confidence limits (Pearson's correlation $r = 0.99$; $p < .0001$).

Fig 7. Cross-sectional area measurement in trachea F, with high-grade stenosis. Comparison between endoscopically measured cross-sectional area and true cross-sectional area was obtained with Cylan 3-D scanner. Passing and Bablok¹ plot was used to compare new measurement method with established one. Dotted lines show linear regression of true values (Cylan scanner). Bullets show endoscopically measured values ($n = 50$). Dashed lines show confidence limits (Pearson's correlation $r = .99$; $p < .0001$).



DISCUSSION

Endoscopy is certainly the best available diagnostic procedure for stenosis, tumors, and ulcers of luminal organs. Unlike computed tomography,²⁻⁵ ultrasound,⁶ and other imaging techniques, endoscopy has not, to date, been susceptible to quantitative evaluation.

The length and planar expansion of a pathological process are of particular interest to surgeons. The grade of stenosis and the spatial expansion of the lesion in relation to anatomic landmarks influence therapeutic decisions. The use of endoscopic examination for endotracheal tube position control and quantification of bronchoconstriction has been proposed.^{7,8} Direct inspection of the airway lumen makes it possible to evaluate the inflammatory status of the mucous membrane and the extent of epithelial lesions and scars. These features, and the advantage of decreased X-ray exposure, sum up the benefits of endoscopy as compared to computed tomography.

With the method presented here, noncontact 3-D measurement of endoscopic findings was achieved. The procedure combines 2-dimensional (2-D) measurement of the cross-sectional area of the stenotic region with registration of the position of the measur-

ing tool in the endoscopically examined cavity. This technique facilitates high-resolution 3-D measurement and graphic display of the internal body surface.

A number of grading systems for airway stenosis have been developed to date. Mechanical instruments have been inserted. Stenosis grading was done in comparison to known dimensions of the inserted materials. Myer et al⁹ used endotracheal tubes of different diameters in evaluation of pediatric stenosis. With the help of mechanical instruments like Kasik's¹⁰ Stenoquantograph, the Olympus Measuring Device, Toohill's endoscopic ruler,¹¹ or the slide caliper introduced by Kleinsasser et al,¹² it was possible to estimate the diameter of short stenoses in some cases. The airway deformities associated with laryngotracheal stenosis as seen in most therapeutic cases are most irregular and have a complex shape when viewed 3-dimensionally, limiting the utility of these methods. The mechanical irritation of the airway mucosa represents a further side effect.

Initial successes in noncontact measurement were achieved by Jones et al¹³ with their method of "photometrics," as well as by Doolin and Strande,¹⁴ Santos et al,¹⁵ Forkert et al,¹⁶ and Dunham and Wolf,¹⁷ using image calibrating techniques. In the method of

RESULTS OF CLINICAL APPLICATION OF ENDOSCAN SYSTEM IN TEN CASES OF DIAGNOSTIC AND THERAPEUTIC TRACHEOSCOPY

Patient	Indication for Endoscopy	Instrumentation	Clinical Applicability
1	Dumon stent implantation	Rigid endoscopy	Very good
2	Tracheal scar control	Flexible endoscopy	Very good
3	Carbon dioxide laser incision	Rigid endoscopy	Difficult
4	Tracheal resection control	Flexible endoscopy	Good
5	Bilateral vocal fold palsy	Flexible endoscopy	Problematic
6	Ultraflex stent implantation	Rigid endoscopy	Very good
7	Ultraflex stent implantation	Rigid endoscopy	Very good
8	Tracheal resection control	Flexible endoscopy	Very good
9	Postintubation stenosis	Flexible endoscopy	Difficult
10	Dumon stent control	Flexible endoscopy	Very good

Jones et al, there is no complete cross section, but only a single point marked by placing a melted Prolene sphere at the narrowest point in the stenosis. The third dimension was also lacking here; it was added by Dunham and Wolf with the restriction of proportionate endoscope movement during examination and the assumption that the pixel-to-millimeter correction factor for the 2-D measurement can be used for the 3-D reconstruction. This method is less precise than direct measurement.

In 1983, Spears et al¹⁸ proposed a method of marking a discrete cross section of coronary arteries in angiography, using a transverse ring of light transmitted through fiberoptics. Independently, our group¹⁹⁻²² and a second team²³ found that a laser ring marking may be the best solution for noncontact measurement of luminal dimensions of the respiratory tract. For the purpose of 3-D measurement and reconstruction, it is necessary to record the current position of the laser ring in the lumen. We solved this problem and combined the 2-D data from the laser ring-based measurement of discrete cross sections with a laser fiber tip position detection system. In this way, genuine 3-D measurement of the size and shape of airway lesions is possible (unpublished observations presented at the 5th International Meeting on Respiratory Endoscopy, Barcelona, Spain, 1997, and at the 10th World Congress for Bronchoesophagology, Budapest, Hungary, 1998). The evaluation of the data can be done in a few minutes on the computer after the patient examination. With a Pearson correlation of $0.97 \leq r \leq 0.99$ between our measuring results and the controls, the accuracy of our measured values is very good.

The clearest advantage of our new measuring technique lies in its unobtrusive handling characteristics; ie, the normal procedure of tracheoscopy is not disturbed. The method proved practical in conjunction with rigid endoscopy, as well as with flexible endoscopy under local anesthesia. We observed no side effects or unpleasantness for the patients. In our clinical experience, the procedure is relatively safe and brief.

One disadvantage we experienced with this technique was a problem with laser ring detection in the poststenotic "shadow," ie, the segment in which the endoscopic field of view is blocked by the stenosis. The endoscope is moved from the larynx in the di-

rection of the carina. The diameter of the prestenotic airway and the stenosis itself, as well as the region after the stenotic shadow, is readily measurable. The shadow problem is experienced only in the short segment immediately following the stenosis and only in stenoses with a rapid change in diameter. The choice of therapy depends on the length, localization, and grade of the stenosis. By the proposed method, these parameters can be determined at levels of accuracy and reproducibility that meet the demands of clinical practice.

CONCLUSIONS

The results achieved with our method of analyzing and evaluating the explored airway improve the examining physician's 3-D grasp of airway lesions. Dynamic changes in luminal dimensions can also be analyzed. For example, it is interesting for the airway surgeon to know the consistency of a stenosis. Brown et al²⁴ investigated the variability in the size of individual airways over the course of 1 year. With the proposed method, objective measurement of the flexibility of the tracheal wall, as well as posttherapeutic changes, becomes feasible.

A higher-quality standard can be achieved in such cases by using the new measuring technique and planning the therapeutic procedure virtually. Tele-medical consultation is also conceivable by means of electronic transfer of endoscopic measurement data.

We see a further use for this method in endoscopic-assisted manufacturing (EAM) of anatomically enhanced stents and cannulas. It could help avoid injuries due to incorrect product dimensions.

Fundamentally, the measurement principles presented here can be applied to other endoscopic applications, as well: pharyngeal obstructions in snoring, stenosis of blood vessels, stenosis of the urogenital tract or the bile duct, and measurement of tumor and ulcer expansion in the gastrointestinal tract. Applications outside the field of human medicine are, of course, also conceivable.

Most important, the endoscopic findings can be documented in an objective and efficient way. The potentials of this method in theoretical and research work are considerable: the pathogenesis of airway disturbances can be described more accurately and vividly to form a basis for better therapeutic concepts.

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DELAYED PRESENTATION OF LYMPHATIC MALFORMATION OF THE CERVICOFACIAL REGION: ROLE OF TRAUMA

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Lymphatic malformations (LMs) are uncommon congenital lesions that may occur throughout the body, although the head and neck region is the most common site. Most LMs are seen at birth. However, they may present in adolescence or adulthood, mainly as a result of trauma or infection. We report the case of a 7-year-old boy who presented with an LM of the cervicofacial region causing airway compression. We discuss the causes of delayed presentation of these congenital lesions. An overview of the causation, natural history, diagnosis, and treatment options is presented.

KEY WORDS — lymphatic malformation, trauma.

INTRODUCTION

Lymphatic malformation (LM) is a term that incorporates all congenital malformations of the lymphatic system and is one of the main differentials in the diagnosis of congenital masses of the head and neck.¹ It is the result of a defect in the embryological development of primordial lymphatic channels.² Lymphatic malformations are seen in various forms, from a localized spongiform lesion to diffuse involvement of a particular region or multiple organ systems. The majority are noted before the age of 2 years. Delayed presentations are often prompted by trauma or infection.

CASE REPORT

A 7-year-old boy was referred to a local hospital with a right-sided neck mass (Fig 1A). He presented after sustaining local trauma to the right side of his neck in a fall, which had caused unexpected neck swelling. The initial line of management was a small incision and drainage procedure for a presumed diagnosis of cervical hematoma. However, the neck swelling reappeared 24 hours after the incision and drainage. Over the next 24 to 36 hours, the neck swelling increased in size, and the patient was referred to our institution for further workup. His examination was significant for fullness in the right side of the neck, extending from the body and angle of the mandible to the clavicle. Fiberoptic laryngoscopy revealed marked medialization of the right lateral pharyngeal wall caused by external compression, and a normal glottis and normal vocal cord function. Mag-

netic resonance imaging and computed tomographic scans showed a mass consistent with a macrocystic LM with mild airway compression (Fig 2). The mass was excised. The operative findings included a 10 × 12-cm mass occupying the entire right side of the neck, extending from the base of the skull to the clavicle (Fig 1B). The sternocleidomastoid muscle was splayed by the mass, and cranial nerves X, XI, and XII and the internal jugular vein were identified and protected. The patient made an excellent postoperative recovery and was discharged home on postoperative day 3. The histologic examination confirmed an LM involving the soft tissue (Fig 3). The mass was composed of multiple large, anastomosing, thin-walled vascular channels lined by a flat endothelium. For the most part, the vascular spaces appeared empty, but sometimes they contained a pink, amorphous and acellular fluid consistent with LM (Fig 3). The patient has been followed for 2 years without any evidence of recurrent disease.

DISCUSSION

Despite the fact that vascular anomalies of the head and neck are common in children, confusion often exists in their nomenclature, classification, diagnosis, and treatment.¹ For example, cystic hygroma, lymphangioma, and LM have been used to describe the same conditions. The term hemangioma is often used to describe various vascular lesions such as strawberry marks, port-wine stains, and capillary hemangiomas, which have different causes and natural

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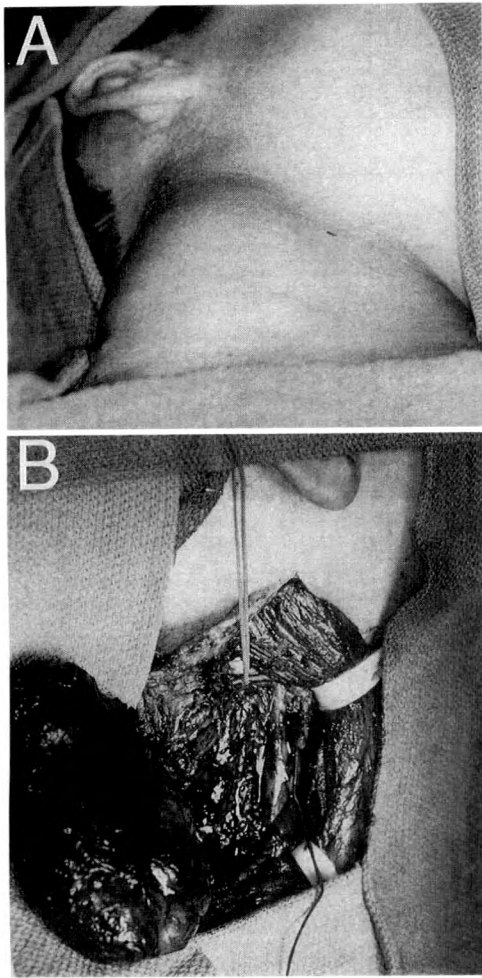


Fig 1. Clinical photographs. A) Preoperative. B) Intraoperative.

histories. In 1982, Mulliken and Glowacki² proposed a biological classification system based on clinical, histochemical, and cellular criteria to distinguish between various vascular anomalies. In this classification system, the cervicofacial vascular anomalies are divided into 2 categories: hemangiomas and malfor-

mations. Vascular malformations are further subdivided into slow-flow and fast-flow ones.²

Hemangioma is the most common vascular tumor, presenting in the skin of 4% to 9% of infants.¹ It is more frequent in girls, with a ratio of 3:1. It occurs more commonly in white infants than in dark-skinned infants. The hallmark of hemangioma is rapid growth (the proliferative phase) followed by slow regression (the involuting phase).

In contrast, LMs present with equal frequency in both sexes and among races. They can be characterized as microcystic, macrocystic, or combined. Histologically, LMs are formed of vascular spaces filled with eosinophilic and protein-rich fluid. There are localized collections of lymphocytes, and the walls of the lymphatic channels have variable thickness and abnormally formed smooth and skeletal muscular elements.¹ All LMs are present at birth. However, the present case highlights the fact that not all LMs are clinically obvious at birth or, indeed, in the first 2 years of life. Furthermore, it demonstrates the role of a relatively minor trauma in bringing LM to clinical attention. Traumatically revealed LM has been reported in different parts of the body, including the head, neck, and ulnar nerve.³⁻⁵ However, the head and neck appears to be the most common location for traumatically revealed LM.⁴ Although the cause is not entirely clear, it appears that trauma may induce changes in the local lymph and blood microcirculation that cause a collection of blood, lymph, and swelling.⁶

Lymphatic malformations of the head and neck usually present as a swelling that may be either superficial or deep and either localized or diffuse. They are generally harder and more difficult to compress than either venous or arteriovenous malformations. Pitting edema is usually present in an LM.⁷ The pre-

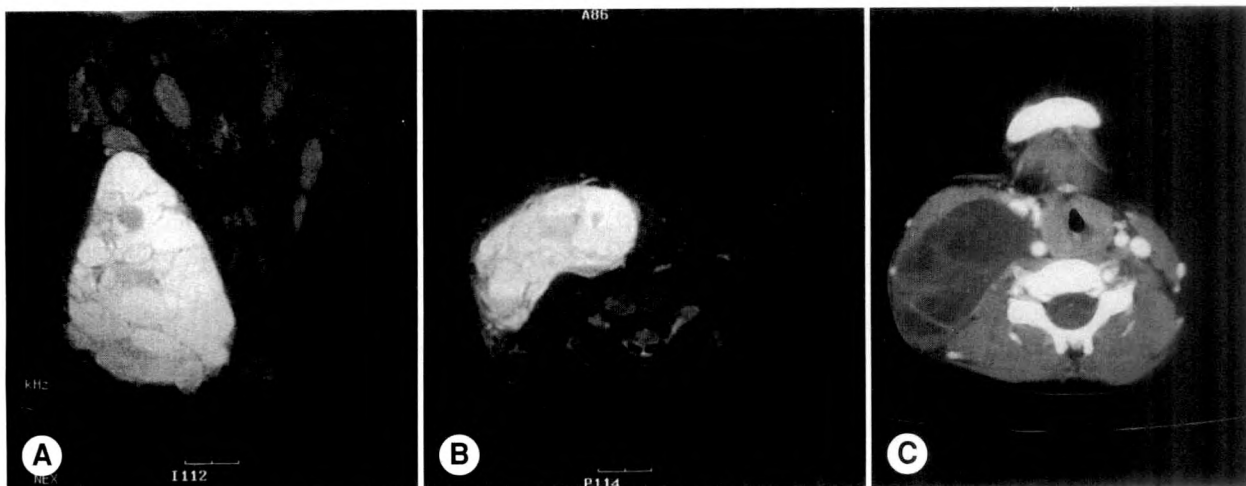


Fig 2. Imaging. A) Coronal magnetic resonance image. B) Axial magnetic resonance image. C) Axial computed tomogram.

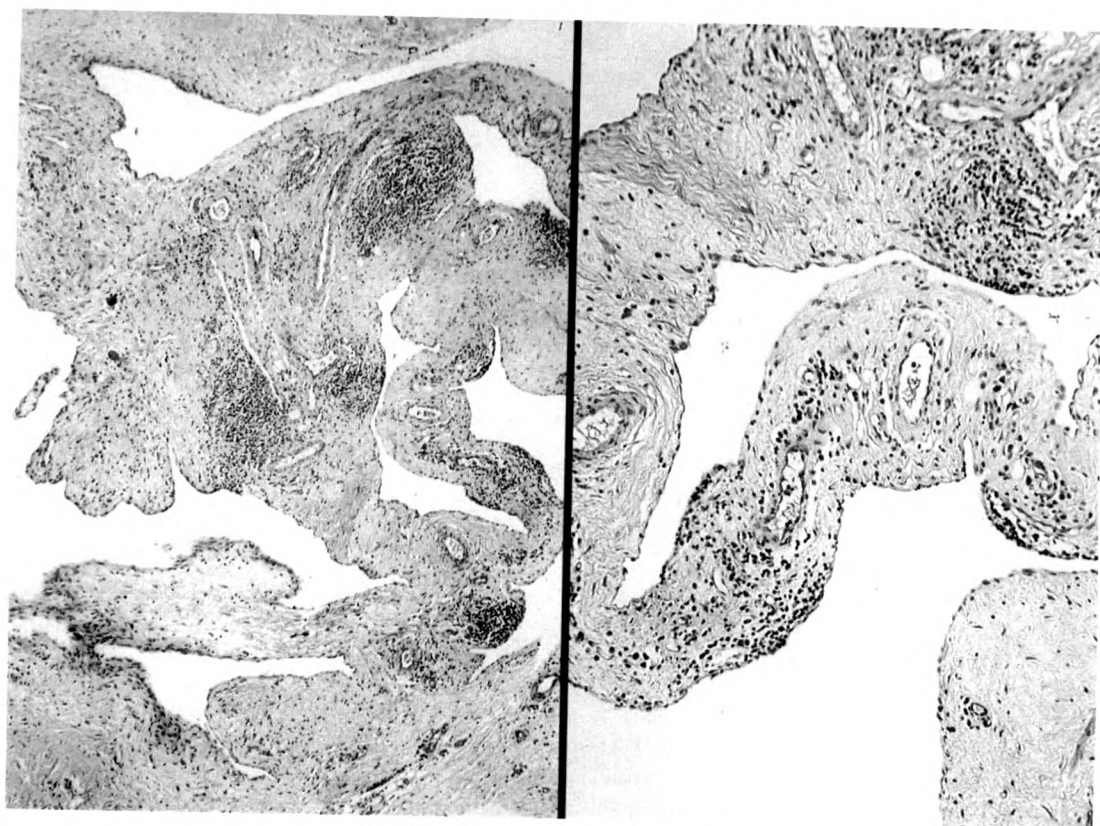


Fig 3. Histologic photomicrographs.

operative investigation of these masses frequently involves either computed tomography or magnetic resonance imaging.⁸ The latter is preferable and will show a hyperintense lesion on T2-weighted sequences because of its high water content. Lymphatic malformations are slow-flow lesions and, unlike venous malformations, do not enhance with gadolinium. Contrast administration usually results in enhancement of the rims of large cysts or septae.

Lymphatic malformations can be divided into 2 categories on the basis of anatomic distribution and imaging and histologic findings. Type I LMs are located below the level of the mylohyoid muscle and involve the anterior and posterior cervical triangles. Histologically, they have large cystic structures without infiltration of the adjacent soft tissue. Type II LMs are found above the level of the mylohyoid muscle and involve the lip, tongue, and oral cavity. These lesions classically have smaller lymphatic channels with infiltration of the surrounding soft tissue.¹ The 2 major complications that may occur with LMs are intralesional bleeding and infection.¹ These can be potentially life-threatening, as they may precipitate airway compromise.

The appropriate management of LMs falls into 2 main categories. Certain macrocystic swellings are suitable for embolization with a lyophilized incubation mixture of group A *Streptococcus pyogenes*

called OK-432.^{9,10} Other LMs, which are microcystic in nature or have undergone recent trauma, are probably best treated surgically. Surgical resection can be challenging if the LM crosses tissue planes and involves the vital structures.^{11,12}

Therapeutic modalities such as aspiration, radiotherapy, and repeated incision and drainage should be avoided. Treatment should be based on the type of LM (ie, microcystic, macrocystic, combined) and the location and extent of the lesion. Surgical excision should be undertaken with the thought in mind that LMs are benign, and vital structures should not be sacrificed. Cold-knife dissection is the technique of choice.¹ Type I lesions are resected fairly easily, and surgical intervention should be 1-stage and can be carried out within the first 9 to 12 months of life.¹ Surgical excision of type II lesions is more challenging and often requires staged resection because these lesions present without any distinct tissue planes. Resection of these lesions before 5 or 6 years of age is recommended.¹ Lasers have a role in the management of lesions of the oral cavity, tongue, and supraglottic region. The CO₂ laser is the technique of choice for lesions of the supraglottic area, and the neodymium:yttrium-aluminum-garnet laser is effective in the treatment of tongue and oral cavity lesions.

The long-term psychological and social ramifications of these malformations should not be underes-

timated. In consideration of surgical resection of LM, issues such as the extent of the lesion, involvement of neural and vascular structures, single or staged resection, and realistic expectations on the part of the

patient should be included. In planning surgical excision, restrictions should be set for the extent of dissection, the duration of surgery, and acceptable blood loss.

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POSTNATAL DEVELOPMENT OF STATIC VOLUME OF THE EUSTACHIAN TUBE LUMEN

A COMPUTER-AIDED THREE-DIMENSIONAL RECONSTRUCTION AND MEASUREMENT STUDY

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Eleven human temporal bone–eustachian tube (ET) specimens obtained from 11 individuals (ages, 3 months to 88 years) were studied to investigate the static volume of the ET lumen. The volumes were measured by computer-aided 3-dimensional reconstruction and measurement methods. Four of the 11 individuals were children, and the remaining 7 were adults. The total volume of the ET lumen was significantly larger in the adults than in the children ($p < .01$). This difference was produced by significant growth of the volume of the cartilaginous and junctional portions ($p < .01$). The lumen of the bony portion, by contrast, did not change with age. Increased volume of the ET lumen, especially of the cartilaginous and junctional portions, would be expected to increase both ventilation and clearance. Therefore, the small volume of ET lumen of the cartilaginous and junctional portions in children may be a predisposing factor for otitis media.

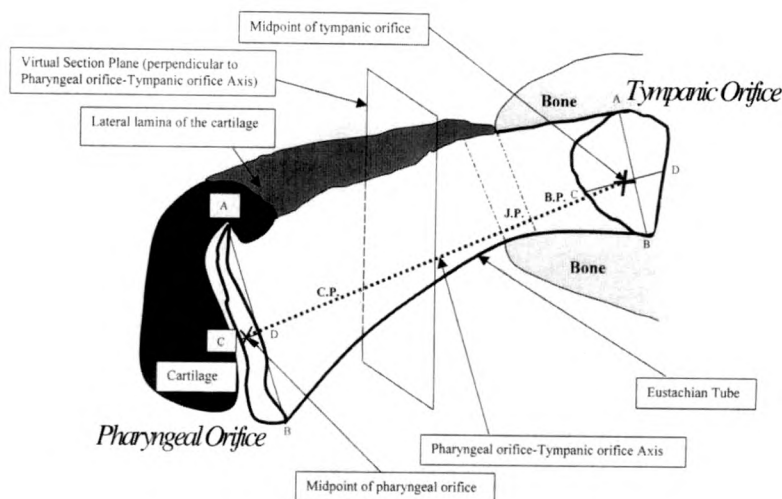
KEY WORDS — computer-aided three-dimensional reconstruction, eustachian tube, postnatal development, volume.

INTRODUCTION

Immaturity of the eustachian tube (ET) has been implicated as an explanation for the high prevalence of otitis media with effusion in children.¹ Our recent studies have revealed that the path length of the ET lumen increases remarkably up to the age of 7 years² and that the shape of the lumen of the cartilaginous

portion of the ET changes markedly during adolescence (ages 10 to 17 years), from a flattened tube to a flattened funnel opening widely at the pharyngeal orifice.³ The purpose of this study was to estimate postnatal developmental changes in the volume of the ET lumen in the static state from a series of histologic sections of human temporal bone–ET specimens.

Fig 1. Schematic drawing of left eustachian tube (ET) viewed from anterosuperolateral direction. Line A-B is long axis of cross sections of ET lumen at its pharyngeal and tympanic orifices. Line C-D is line perpendicular to and bisecting line A-B. Midpoints (X) of line C-D identify centers in each orifice of ET lumen. Pharyngeal orifice–tympanic orifice axis is drawn by connecting midpoints of cross sections of both pharyngeal and tympanic orifices. Virtual cross sections are made perpendicular to axis from pharyngeal end to tympanic end. Pharyngeal orifice was defined as anteriormost virtual cut section containing complete cross section of mucosal lining of ET; tympanic orifice was defined similarly with respect to anterior-superior margin of tympanic annulus. Anterior and posterior parts of ET lumen are covered by cartilage and bone, and are called cartilaginous portion (C.P.) and bony portion (B.P.), respectively. Intermediate region, “junctional portion” (J.P.), is covered partly by bone (in inferior part) and partly by cartilage (lateral lamina; in superior part).



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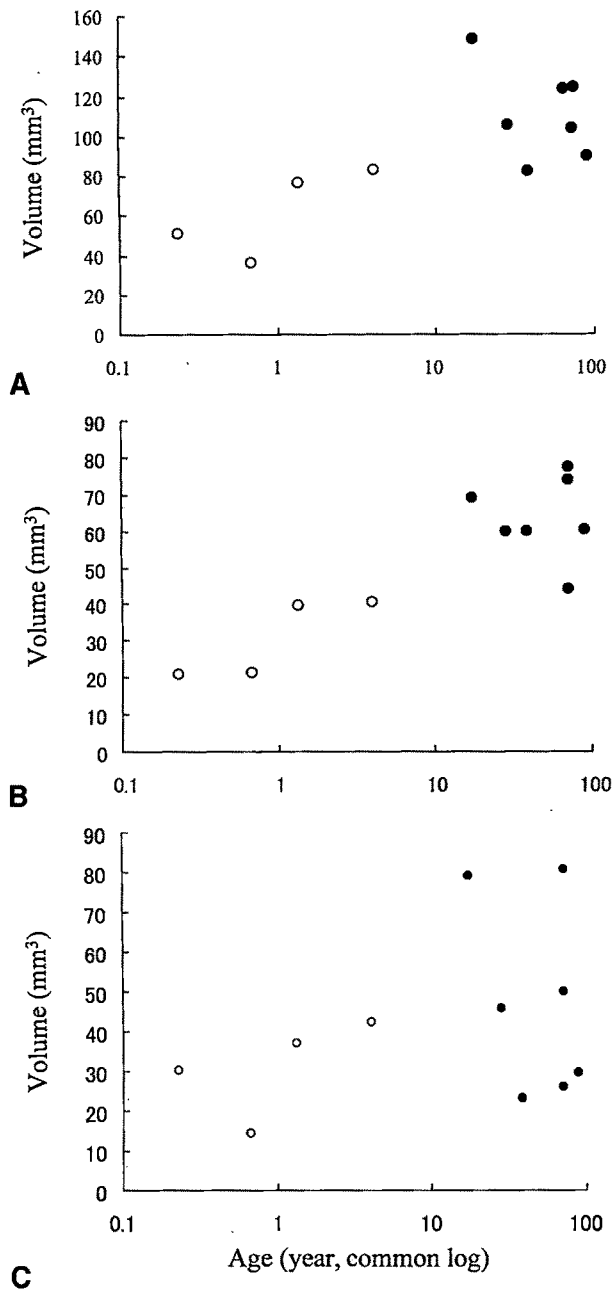


Fig 2. Graphs show data in static condition for each individual. White circles — children; black circles — adults. **A)** Total volume of ET. **B)** Volume of cartilaginous and junctional portions of ET lumen. **C)** Volume of bony portion of ET lumen.

MATERIALS AND METHODS

The materials used for this study were 11 temporal bone-ET specimens obtained from 11 individuals. Their ages were 3 months, 8 months, 16 months, 4 years, 17 years, 28 years, 38 years, 70 years, 70 years, 71 years, and 88 years at death. These individuals did not have any known congenital anomaly or clinical history or histologic evidence of ear disease. We separated these individuals into 2 groups: the child group (4 cases, ranging from 3 months to 4 years old) and the adult group (7 cases, ranging from 17 to

VOLUME OF EUSTACHIAN TUBE IN STATIC CONDITION

Group	n	Cartilaginous Portion With Junctional Portion	Bony Portion	Total Lumen
Child	4	30.74 ± 11.03*	31.23 ± 12.26	61.97 ± 22.27†
Adult	7	63.52 ± 11.13*	47.78 ± 24.01	111.3 ± 22.55†

Data are average ± SD in square millimeters.
 *p < .01 between 2 values so marked.
 †p < .01 between 2 values so marked.

88 years old; mean age, 54.6 years). The specimens were fixed in 10% formalin, decalcified in 5% trichloroacetic acid, dehydrated in graded concentrations of ethanol, and embedded in celloidin.⁴ After reference marks had been made in the celloidin blocks for 3-dimensional (3-D) reconstruction, the blocks were sectioned at 30-μm thickness as close to perpendicular to the axis of the ET as possible. Every 40th section was stained with hematoxylin and eosin and prepared for study by light microscopy. Additional slides between every 40th section were made for the part of the ET between the anterior margin of its junctional portion⁵ and the posterior margin of the bony portion to determine the exact location of both the junctional and bony portions along the entire ET. The junctional portion of the ET was defined as a portion that was located between the bony and cartilaginous portions and was surrounded in part by bone and in part by cartilage.⁵ These histology slides were then used for computer-aided 3-D reconstruction. The equipment used for reconstruction consisted of a personal computer, a frame buffer for full-color images (Sapience HyperFrame+), a digitizer (Hitachi HDG-1515; resolution 0.1 mm), and a printer. With the software developed by Takagi and Sando,⁶ modified by Fujita and Sando,⁷ and revised by Sudo et al,⁵ accurate 3-D reconstruction and measurements were made of the ET lumen.

The ET lumen, the ET cartilage, and the temporal and sphenoid bones bordering the ET were reconstructed for each specimen. The initial step in the analysis of measuring ET volume was identification of a line connecting the centers of the pharyngeal and tympanic orifices (Fig 1). The pharyngeal orifice was defined as the anteriormost section containing a complete cross section of the mucosal lining of the ET; the tympanic orifice was defined similarly with respect to the anterior margin of the tympanic annulus. The center of each orifice was defined as the midpoint of a line bisecting and perpendicular to the line connecting the superior and inferior margins of the lumen. This pharyngeal orifice-tympanic orifice line provides a standard axis for creating virtual

cross sections of the ET lumen perpendicular to the axis through the reconstructed images.

A series of 200 virtual cross sections was used to measure the volume of the lumen of 1) the bony portion of the ET and 2) the combined cartilaginous and junctional portions of the ET. The junctional and cartilaginous portions were regarded as a single functional unit, because the lateral lamina of the ET cartilage extends throughout the junctional region.

RESULTS

The total volume of the ET lumen in each individual is shown as a function of age in Fig 2A. Similarly, the volumes of 1) the cartilaginous and junctional portions of the ET and 2) the bony portion of the ET are shown in Fig 2B and Fig 2C, respectively. Statistical analysis revealed that both the total volume and the volume of the cartilaginous and junctional portions of the ET lumen were significantly larger in adults than in children (see Table, Student's *t*-test, respectively: $t = 3.51$, $p < .01$; $t = 4.71$, $p < .01$). The luminal volume of the bony portion of the ET did not differ significantly between groups (see Table).

DISCUSSION

This, to our knowledge, is the first quantitative morphometric study of the volume of the subdivisions of the ET lumen performed with computer-aided 3-D graphic reconstruction and measurement methods. These measurements from temporal bone specimens are assumed to reflect static dimensions of the lumen.

The most important finding in this study is that the increase in volume of the ET lumen that occurs during development reflects significant growth of the cartilaginous and junctional portions. This result is consistent with previous studies that found that the path length of the ET lumen increases during development² and that the shape of the lumen of the cartilaginous portion of the ET changes markedly during development, from a flattened tube to a flattened funnel opening widely at the pharyngeal orifice.³ This volumetric change (enlargement) that occurs during

development is likely to increase the clearance function of the cartilaginous and junctional portions of the ET, because ciliated cells,⁸ goblet cells⁹ and tubal glands are richly distributed in these portions.

By contrast, the volume of the bony portion of the ET lumen did not change significantly. This finding likely reflects the fact that the mesotympanum of the middle ear cavity (which extends directly to the bony portion of the ET) shows little postnatal growth, despite obvious increases in the volume of the hypotympanum and the epitympanum.¹⁰

Morphological changes of the ET lumen, such as changes in path length² and shape,³ have been considered to be important factors in the reduced prevalence of otitis media during adolescence. From a clinical viewpoint, it is suspected that inflammation may render the ET lumen more collapsible.¹¹ When inflammation is present, an ET lumen with a small volume may collapse more easily than one with a large volume. Therefore, a small volume of the ET lumen in children may be one of the predisposing factors of otitis media.

Sadler-Kimes et al,¹² using a 3-D computer graphic technique, reported that the ET lumen volume is $30 \pm 10 \text{ mm}^3$ in subjects younger than 7 years of age and $40 \pm 30 \text{ mm}^3$ in subjects older than 7 years of age. The most important factor causing discrepancies between our results and their results is probably the different extent of the reconstruction of the ET lumen. Because they produced reconstructive data from histologic sections containing the ET cartilage, their results may be consistent with the lumen volume of the cartilaginous and junctional portions of the ET. Other factors, including different definitions of the ET orifices, methods of measurement, and age distributions of subjects, may also contribute to the discrepancy. Unavoidable shrinkage artifact in the specimens¹³ produced by histologic processing could cause a consistent underestimation of the volume of the ET lumen. However, it cannot reasonably be assumed that this artifact would essentially affect the data on development of the volume of the ET lumen in the static condition.

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ULTRASONOGRAPHY IN A NASAL CAVITY FILLED WITH WATER DURING ENDOSCOPIC SINUS SURGERY

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We developed a new ultrasonographic imaging technique to be used in a water-filled nasal cavity during endoscopic sinus surgery, and estimated how well surgically dangerous sites were depicted. Our newly developed balloon can completely fill the choana, preventing water from leaking into the pharynx. The balloon enables endoscopic sinus surgery to be relatively safely performed in "flowing water." Fifteen patients with chronic sinusitis underwent endoscopic sinus surgery under local anesthesia, and a MicroTip Radial probe was inserted into the water-filled nasal cavity. The orbital contents, skull base, and maxillary sinuses were visualized in all cases. Even in cases with a thin lamina papyracea, the boundary of the orbit could be judged from the motion of the orbital contents. Ultrasound information obtained in real time will be useful for improving the safety of endoscopic sinus surgery. Our ultrasonographic imaging technique can provide an early warning to the surgeon and thereby prevent severe complications.

KEY WORDS — complication, endoscopic sinus surgery, flowing water, Noda balloon, ultrasonography.

INTRODUCTION

Image-guided surgery systems, such as an electromagnetic tracking system,¹ an optical digitizing system,² and a mechanical arm system,^{3,4} have recently been reported in the literature as useful technologies for endoscopic sinus surgery (ESS). These computer-assisted localization methods allow surgeons to have confidence and knowledge of patient anatomy, and would be effective in preventing complications associated with surgery. Here we propose another imaging system different from these navigation systems: ultrasonography of the sinus.

Ultrasonography of the sinus has been depicted thus far only by using sensors on the face.⁵ It has, so far, been difficult to obtain a clear image of the sinus by using ultrasonography, because air in the nasal cavity does not conduct ultrasound. However, our newly developed balloon,^{6,7} named the "Noda balloon," can perfectly fill the choana and enables the nasal cavity to be relatively safely filled with water, making ultrasonography available in the nasal cavity.

The Noda balloon prevents water from leaking into the pharynx, even when water is flowing into the nasal cavity at a rate of 1 L/min. Therefore, we can perform ESS even in "flowing water."^{6,7} This surgical technique is similar to that used in transurethral resection of the prostate. The tip of the endoscope is kept clean, and blood, debris, and resected tissues are continuously removed by the water flow.^{6,7}

This unique method still has some topics for dis-

cussion, such as its complications, merits, and disadvantages. We, however, believe this technique actually has some possibility for nasal treatment. In the present study, we focused on the availability of ultrasonography. The visual information obtained in real time will allow a safer and more efficient surgical procedure during specific phases of the surgery.

MATERIALS AND METHODS

Noda Balloon. A Noda balloon (Fig 1) was inserted from the nostril into the choana before or during surgery. After expansion of the balloon, the tube of the balloon was stretched out of the nostril and was fastened with a clip near the nostril.^{6,7}

Drainage. A 3-L water pack (like that used for transurethral resection of the prostate) was connected to the flexible tube that ran parallel to the endoscope. The end of the tube was open adjacent to the tip of

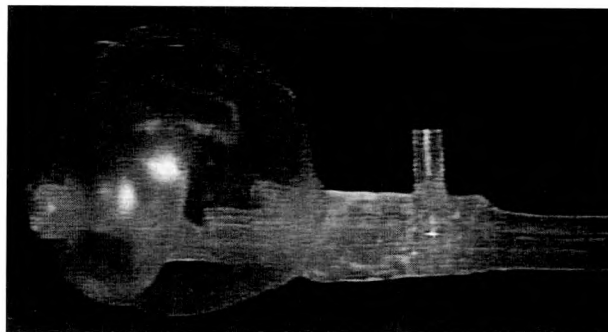
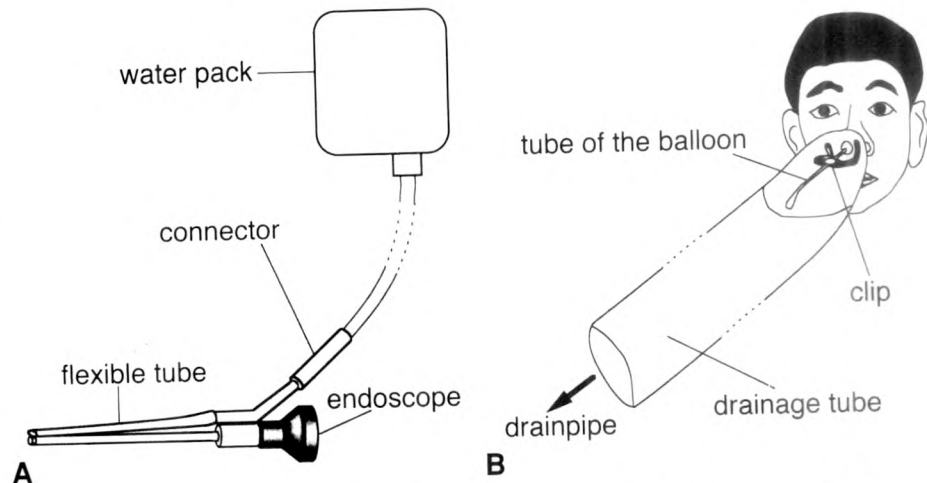


Fig 1. Noda balloon (Koken, Inc, Tokyo, Japan).

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Fig 2. Drainage. **A)** Water is poured from 3-L water pack through flexible tube. **B)** Water flows from nostril and is gathered with drainage tube. (Reprinted.⁷)



the endoscope (Fig 2A).^{6,7}

A jet of water was poured through the tube into the nasal cavity; it cleaned the tip of the endoscope and washed out blood and debris from the surgical field. Then, water overflowed from the nostril into the drainage tube, one end of which was glued on the face and the other end of which was led into a drainpipe (Fig 2B).^{6,7}

Water flow is actually required not to obtain ultrasonographic images, but to obtain clear endoscopic visual images. Especially in Japan, most cases of sinusitis are relatively severe, and bloody water easily disturbs the visual field in such cases. Water flow of 1 L/min has enough power to make any visual field clear, even in cases of severe bleeding. With a Noda balloon, 1 L/min of water flow can be used relatively safely, and much slower water flow usually has enough effect to obtain clear visual fields.

We should emphasize that we do not use the water-flow system during the whole ESS procedure. We use the water-flow system only when we want to use ultrasonography, when we wash the surgical field, or when we want to suppress severe bleeding during surgery. We use 3 to 9 L of water (mean, 3.6 L). To perform the whole ESS procedure in water flow would require much more water and might cause complications, such as hyponatremia as caused in transurethral resection of the prostate. We have performed 40 cases of ESS partially in water flow, including 15 cases in which we also used ultrasonography.

Ultrasound Diagnostic Equipment. We used ultrasound diagnostic equipment (SSD-2000 and Micro-Tip Radial [ASU-101]; Aloka, Ltd, Japan) to examine (10 MHz) the state around surgical sites. This system features a catheter-style radial probe, 2 mm in diameter, that is designed to obtain 360° B-mode ultrasound images.⁸ The probe is easily inserted from the nostril into small cavities because of its flexibility (Fig 3).

Patients. Ultrasonographic diagnosis and ESS were performed under local anesthesia in 15 patients (8 men and 7 women, 42 to 68 years of age) with chronic sinusitis. They gave informed consent before surgery.

RESULTS

General Cases. Immediately after we inserted the ultrasound probe into the nasal cavity, the nasal septum and the inferior turbinate were first visualized in coronal images (Fig 4A). When the probe went into the middle meatus, the middle turbinate also appeared (Fig 4B). As it went more posterior, the bones of the inferior and middle turbinates and the wall of the orbit were clearly imaged (Fig 4C). We observed the motion of the orbital contents when the patients moved their eyes.

In the ethmoid cells, ultrasonography demonstrated that the images of small bones were scattered (Fig 4D). On arrival around the skull base, a ring image of the bone appeared (Fig 4E). The ring seemed to represent a hollow of the skull base. The thick-

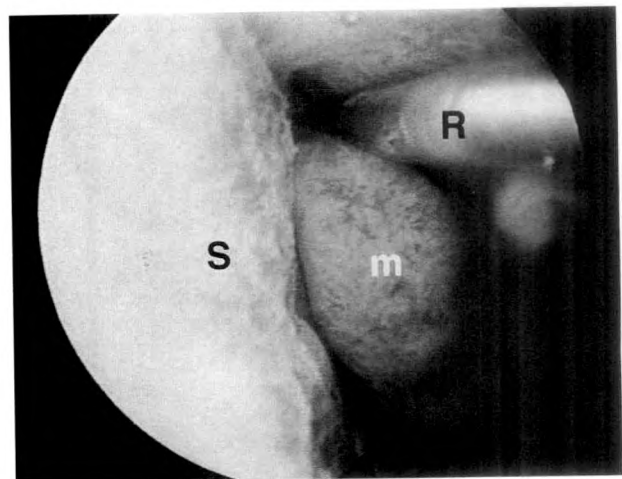


Fig 3. Catheter-style radial probe (R) is inserted in left nasal cavity filled with water. S — nasal septum; m — middle turbinate.

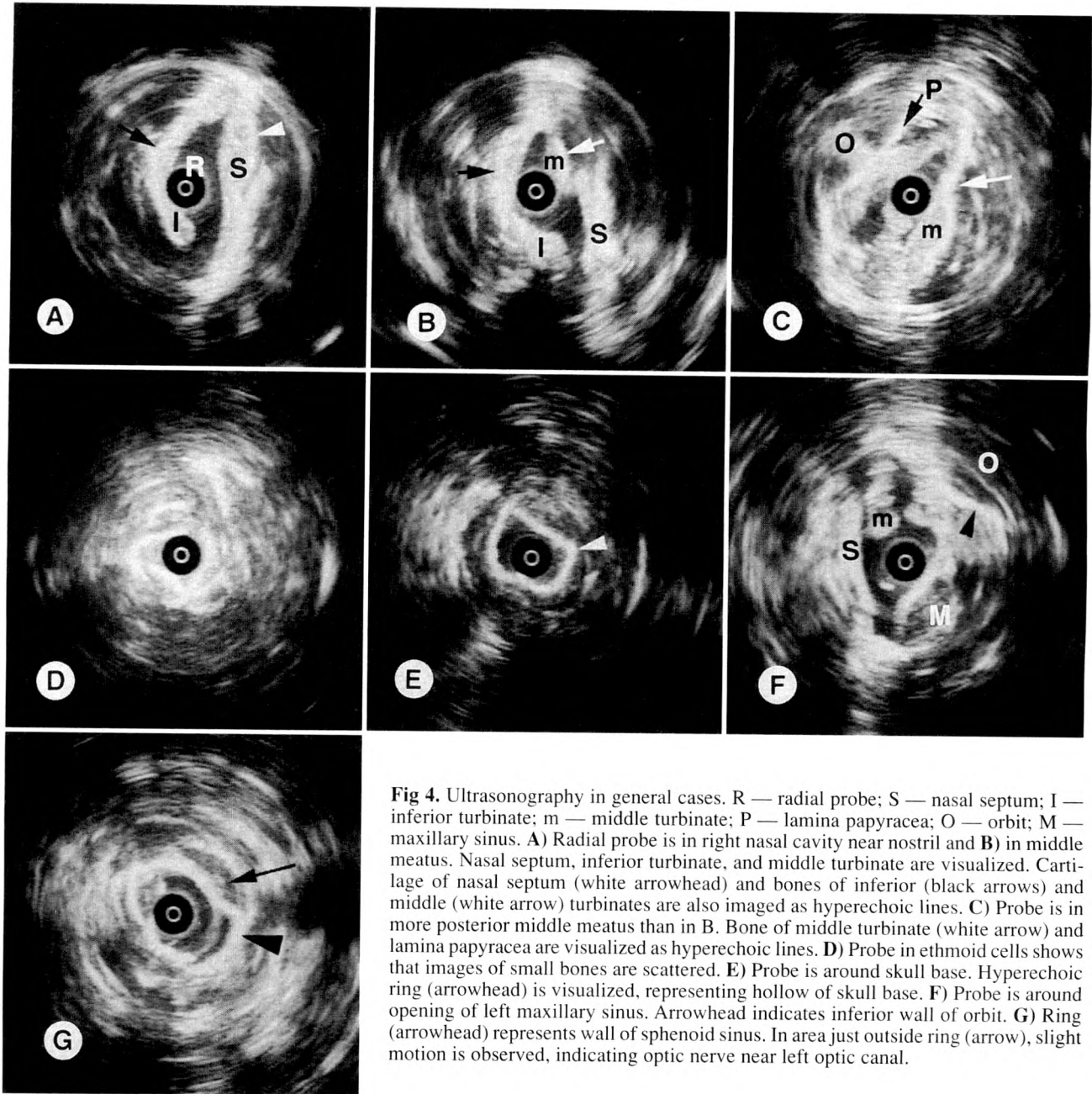
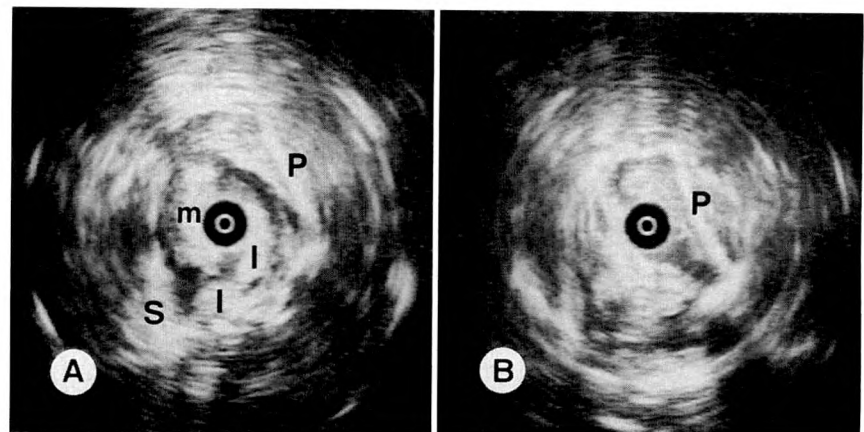


Fig 4. Ultrasonography in general cases. R — radial probe; S — nasal septum; I — inferior turbinate; m — middle turbinate; P — lamina papyracea; O — orbit; M — maxillary sinus. **A)** Radial probe is in right nasal cavity near nostril and **B)** in middle meatus. Nasal septum, inferior turbinate, and middle turbinate are visualized. Cartilage of nasal septum (white arrowhead) and bones of inferior (black arrows) and middle (white arrow) turbinates are also imaged as hyperechoic lines. **C)** Probe is in more posterior middle meatus than in **B**. Bone of middle turbinate (white arrow) and lamina papyracea are visualized as hyperechoic lines. **D)** Probe in ethmoid cells shows that images of small bones are scattered. **E)** Probe is around skull base. Hyperechoic ring (arrowhead) is visualized, representing hollow of skull base. **F)** Probe is around opening of left maxillary sinus. Arrowhead indicates inferior wall of orbit. **G)** Ring (arrowhead) represents wall of sphenoid sinus. In area just outside ring (arrow), slight motion is observed, indicating optic nerve near left optic canal.

Fig 5. Probe is in **A)** middle meatus and **B)** ethmoid cells. Thick lamina papyracea (P) is imaged as hyperechoic line in 58-year-old man with left chronic sinusitis. I — inferior turbinate; m — middle turbinate; S — nasal septum.



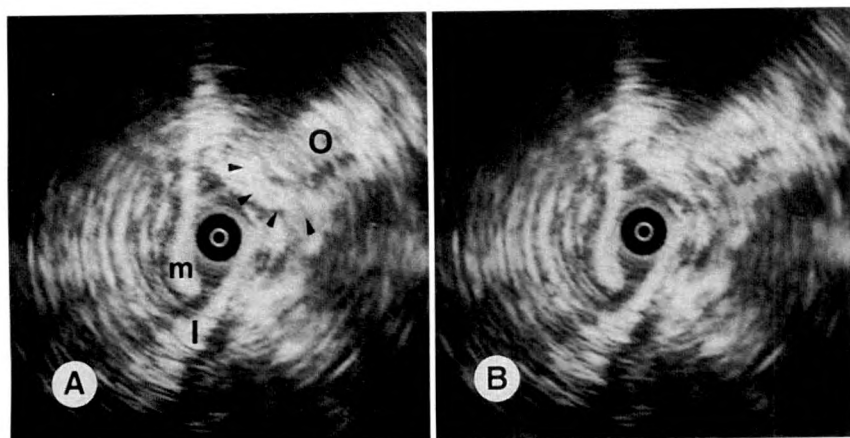


Fig 6. Image of lamina papyracea is unclear in 64-year-old woman with left-sided chronic sinusitis. Boundary of orbit (arrowheads) is distinguishable because of orbital motion (from A to B) correlated with eye movements. I — inferior turbinate; m — middle turbinate; O — orbit.

ness of the line of the ring seemed to reflect that of the bone of the skull base.

When the probe was around the opening of the maxillary sinus, the sinus and the orbit were plainly distinguishable by the image of the inferior wall of the orbit (Fig 4F). Therefore, we could relatively safely approach the maxillary sinus. The opening of the maxillary sinus was imaged as a membrane thinner than the peripheral regions. Around the optic canal in the sphenoidal sinus, a ring was imaged representing the wall of the sphenoidal sinus, and a slight motion of the optic nerve was observed just outside the ring (Fig 4G).

Cases With Thick Lamina Papyracea. The lamina papyracea was clearly demonstrated as a hyperechoic line (Fig 5). We could even observe the motion of the orbital contents according to the eye movement.

Cases With Thin Lamina Papyracea. The lamina papyracea was not always visualized, but the orbital contents were clearly seen, especially through the thinner sites of the lamina papyracea, because of less acoustic shadow.⁹ Although the lamina papyracea was unclear, the boundary of the orbit was definitely judged from its motion when the patient moved his or her eyes (Fig 6).

Case With Defect at Lamina Papyracea. The orbit can be especially well depicted in cases with extensive defects at the lamina papyracea (Fig 7).

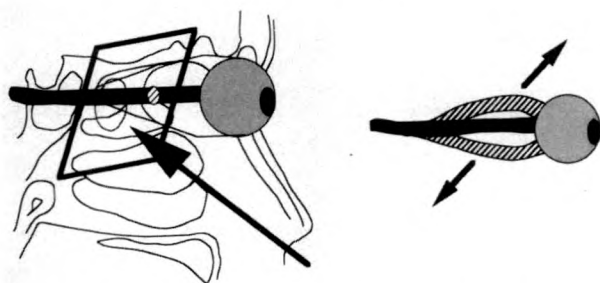


Fig 7. Defect at lamina papyracea enables optic nerve and its motion to be clearly visualized.

In a case with a large cyst of the left medial orbit, ultrasonography depicted the orbit and the optic nerve. Under local anesthesia, eye movements can rapidly alter the position of the optic nerve, and ultrasonography clearly detected its motion (Fig 8).

DISCUSSION

Functional ESS is one of the most commonly performed procedures in otorhinolaryngology,¹⁰⁻¹⁴ but it is always potentially hazardous, because of the proximity of the orbit and skull base.¹²⁻¹⁶ Computer-assisted surgery would be one effective method to minimize the risk. On the other hand, the present study showed that ultrasonography had the capability to decrease significant complications associated with surgery. In order to use ultrasonography, we should consider complications caused by filling the nasal cavity with water. Even though we use this system for a short period, possible complications are 1) water leakage into the pharynx, 2) obstruction of the airway, and 3) discomfort of the throat.

Water leakage into the pharynx occurred in 2 of the 40 cases. In these cases, patients informed us of a mild complaint of water leakage. The amount of the water leakage was less than the amount of blood flowing into the pharynx in the usual method of ESS. When we performed the usual method of ESS (without water flow) using this balloon packing, we frequently recognized that the nasal cavity could easily be filled with blood during surgery. Perfect packing of the choana reveals how much blood patients swallow during the usual method of ESS under local anesthesia. Under local anesthesia, at least, water leakage is not so important. Unfortunately, we have no experience with ESS under general anesthesia. The possible complication of obstruction of the airway should be considered under circumstances in which we must leave the balloon in the nasal cavity even after surgery because of excessive bleeding. If the balloon drops into the pharynx, it might obstruct the airway. We usually use the balloon only during sur-

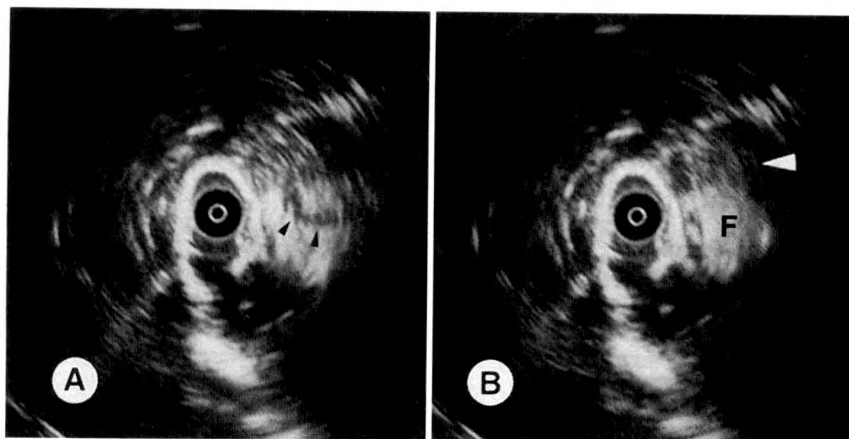


Fig 8. A) Optic nerve can be noted as hypoechoic thick line (arrowheads) within white hyperechoic area representing retrobulbar fat in 52-year-old man with extensive lack of left lamina papyracea. B) When patient looks to his right, optic nerve moves to his left and disappears. Arrowhead indicates approximate site of optic nerve (not seen). F — retrobulbar fat.

gery, and no case of obstruction was seen among the 40 cases. Throat discomfort is not of major concern, because only 1 case was seen among the 40 cases.

We could recognize the boundaries of the orbits in both cases with thin (Fig 6) and thick (Fig 5) laminae papyraceae by the motion of the orbit and by the bone images themselves, respectively. This outcome indicates that we can avoid orbital complications, especially under local anesthesia, and in the future, with accumulated data on ultrasonography, we will also be able to estimate the accurate boundary of the orbit under general anesthesia.

Unfortunately, ultrasonography is not very useful for localizing structures and guiding the surgeon to distant tumors or cysts located behind thick bones, because hard masses or bones absorb ultrasound. In this situation, navigation systems are more appropriate than ultrasonography. Nevertheless, ultrasonography can clearly demonstrate surgical sites, especially during crises, which include cases with a thin

lamina papyracea, with a fractured skull base, or with exposed dura mater. Ultrasonography can provide early warning to the surgeon and thus prevent severe complications.

Ultrasonography also has several practical advantages over navigation systems: 1) it is less expensive than navigation systems; 2) preoperative examinations such as computed tomography are unnecessary; 3) visual information can be obtained in real time; and 4) the error of the device location measurement (estimated to be about 2 mm in navigation systems¹⁻⁴) is not a consideration. Therefore, we can relatively easily use ultrasonography to avoid complications in most surgeries for chronic sinusitis.

The closer a crisis comes, the clearer are the images demonstrated by ultrasonography. For example, orbital contents can be well depicted in a case with a thin lamina papyracea. Ultrasonography will be a highly useful method for performing safer ESS procedures.

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TRAVELING FELLOWSHIP

The Department of Otolaryngology–Head and Neck Surgery at the Hospital Europeen Georges Pompidou, University of Paris V, France, together with the association Progres 2000, is sponsoring a traveling fellowship starting May 1, 2003. A 2-month scholarship will provide extensive training in laryngology–head and neck surgery and oncology every year during the months of May and June. The successful candidate will be an active member in the department and participate in research. For further information, contact ollivier.laccourreye@hop.egp.ap-hop-paris.fr.

IMPACT OF ACTIVITIES OF Na^+ , K^+ -ATPASE AND Ca^{2+} -ATPASE IN THE COCHLEAR LATERAL WALL ON RECOVERY FROM NOISE-INDUCED TEMPORARY THRESHOLD SHIFT

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The present study was designed to investigate the relationship between the noise-induced temporary threshold shift (TTS) and the specific activities of sodium potassium adenosine triphosphatase (Na^+ , K^+ -ATPase) and calcium adenosine triphosphatase (Ca^{2+} -ATPase) in the cochlear lateral wall. The specific activities of these enzymes were quantified by microcolorimetric assay. Changes in auditory brain stem response (ABR) thresholds were compared with the quantitative alterations of the specific activities of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall of guinea pigs with a noise-induced TTS. In the majority of those noise-exposed ears with complete recovery of ABR thresholds, the specific activities of both enzymes returned to at least 70% of the mean specific activity of the control group. Although other factors may be involved, reversible inactivation of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall may be one component of the TTS. Our present findings could drive further studies on the molecular basis of noise-induced hearing loss.

KEY WORDS — Ca^{2+} -ATPase, Na^+ , K^+ -ATPase, noise, temporary threshold shift.

INTRODUCTION

The phenomenon of noise-induced temporary threshold shift (TTS) has been studied for many years. There have been many suggested mechanisms for TTS, including synaptic fatigue,¹ metabolic fatigue of either the stria vascularis or hair cells,^{2,3} changes in cochlear blood flow,⁴ reversible morphological changes of hair cells,⁵⁻¹³ and nondetectable morphological disruption of the mechanoelectrical channel.^{14,15} Changes of enzymatic activities associated with metabolic damage within the cochlea in acoustic injury have not been sufficiently investigated. Multiple energy-consuming ion transport processes in the stria vascularis are involved in the maintenance of the endocochlear potential and normal auditory function.¹⁶ Sodium potassium adenosine triphosphatase (Na^+ , K^+ -ATPase) participates in the active sodium ion (Na^+) and potassium ion (K^+) transport in the inner ear and plays an important role in maintaining cochlear function.¹⁶⁻¹⁸ Calcium adenosine triphosphatase (Ca^{2+} -ATPase) participates in the calcium ion (Ca^{2+}) transport system for the regulation of Ca^{2+} concentrations in the inner ear and influences the mechanoelectrical transduction process in the cochlea.^{11,19-23} High activities of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase have been shown in the cochlear lateral wall by enzyme cytochemical studies, immu-

nocytochemical studies, and biochemical measurements.^{17,18,21,23-28} The functional role of the cochlear lateral wall is presumed to be the maintenance of the unique ionic composition of the endolymph and the intracellular ion content, both of which are crucial to cochlear function.

Metabolic damage to noise-exposed cochleas had not been revealed by quantitative studies of enzyme-specific activity until our recent study.²⁹ In that study, in noise-exposed guinea pigs with TTS, detailed chronological alterations of these enzyme activities in the cochlear lateral wall were not investigated. Because the onset and recovery of noise-induced TTS may follow a complex time course, the exact relationship between the quantitative activities of these enzymes in the cochlear lateral wall and the recovery of auditory function still remains unclear for noise-induced TTS. In this study, the threshold of the auditory brain stem response (ABR) and the levels of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase specific activities in the cochlear lateral wall were measured in guinea pigs with noise-induced TTS at a variety of survival times. The microcolorimetric assay method, which allows quantification of enzyme activity in the cochlear lateral wall from a single cochlea, was used to determine the specific activities of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase.

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TABLE 1. MEAN AUDITORY BRAIN STEM RESPONSE THRESHOLDS OF VARIOUS NOISE-EXPOSED GROUPS AND CONTROL GROUPS

Group	n	Survival (d)	Auditory Brain Stem Response Thresholds		
			a	b	c
N-1	10	0	27.00 ± 4.22	44.50 ± 10.66*	44.50 ± 10.66†
N-2	10	1	26.00 ± 3.94	41.00 ± 6.99*	33.00 ± 6.32†
N-3	10	2	22.50 ± 3.54	41.50 ± 7.47*	26.50 ± 5.30‡§
N-4	10	3	28.50 ± 2.42	41.00 ± 8.43*	28.50 ± 4.74‡§
N-5	10	7	26.50 ± 2.42	43.50 ± 8.18*	27.00 ± 2.58‡§
N-6	12	28	29.58 ± 1.44	46.67 ± 10.08*	28.75 ± 2.26‡§
C-1	10	0			27.00 ± 3.50
C-2	8	28	26.90 ± 5.30		24.40 ± 4.96

Data are mean ± SD in decibels sound pressure level.

N — noise-exposed; C — control; a — initial measurement made before noise exposure; b — measurement made immediately after noise exposure; c — final measurement made before death.

*Significant difference between a and b ($p < .01$; t -test).

†Significant difference between a and c ($p < .01$; t -test).

‡No significant difference between a and c ($p = .06$, $p = 1.00$, $p = .66$, and $p = .29$; t -test).

§No significant difference between noise-exposed groups and control group (all controls pooled together as 1 control group) at termination ($p = .81$, $p = .38$, $p = .97$, and $p = .19$; t -test).

MATERIALS AND METHODS

In total, 40 healthy, adult male Hartley albino guinea pigs with normal hearing, weighing 250 to 300 g, were used. The thresholds of ABRs measured before the experiment ranged from 20 to 30 dB sound pressure level (SPL) for all ears of animals included in this study. Thirty-one guinea pigs were subjected to a single continuous exposure of broadband white noise at around 105 ± 2 dB SPL for 10 minutes to induce a TTS, and these were divided into 6 groups to allow for various recovery periods, as described in Table 1. During noise exposure, the animals were kept in a cage inside a soundproof room. White noise was produced by an audiometer (Grason-Stadler, model GSI-10, Milford, New Hampshire) and presented through a set of loudspeakers at both sides of the cage in which the animals were kept. The SPL of the noise was measured with a sound level meter (Nion, model NA-24, Tokyo, Japan). The remaining 9 guinea pigs not exposed to noise served as controls. In order to rule out an effect of aging, the controls were divided into 2 groups with a difference in survival time of 28 days. The animals used in this study were 4 weeks old.

An evoked potential system (Nicolet, Spirit, Madison, Wisconsin) was used to measure the thresholds of the ABR in all animals. Click sounds (57.7/s) were used to evoke the ABR. The intensity of the stimulus was varied in 5-dB stepwise increments. The response signals were recorded by subcutaneous needle electrodes. The active electrodes were placed in the vertex and the ipsilateral retroauricular region with a ground electrode on the neck of the animal. Animals of the nonexposed control group C-1 were painlessly sacrificed after measurement of ABR thresh-

olds. The ABR thresholds were measured again in the nonexposed control group C-2 just before sacrifice after a survival time of 28 days. In all noise-exposed groups, ABR thresholds were measured immediately after noise exposure and later again just before sacrifice. The extent of auditory dysfunction in experimental guinea pigs was assessed by determining differences of ABR thresholds between preexposure and postexposure or termination.

Immediately after the final ABR measurement, the animals were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg body weight) and decapitated. After decapitation, the temporal bones were quickly removed, opened to expose the otic capsule, quick-frozen by immersion in liquid nitrogen, and then stored at -70°C until microdissection. The stria vascularis and the spiral ligament of the lateral wall of individual cochlea were harvested as a unit, collected in 0.6 mL of dilution buffer (mannitol 310 mmol/L, HEPES 25 mmol/L) in a 2-mL microcentrifuge tube, and stored at -10°C until assay. Efforts were made to avoid contamination with other cochlea tissues such as the organ of Corti, spiral limbus, and Reissner's membrane. Lateral wall tissue was discarded if contaminated by pieces of other tissues.

The microassay procedure was described previously.²⁹ The assay protocol used a total assay volume of 120 μL (10 μL of sample or standard, 10 μL of 0.1N sodium hydroxide [NaOH], and 100 μL bicinchoninic acid) to determine protein content. Bovine serum albumin (BSA) was used as the standard. The linearity of the standard curve was confirmed. Optical density (OD) was determined at 570 nm by an enzyme-linked immunosorbent assay (ELISA)

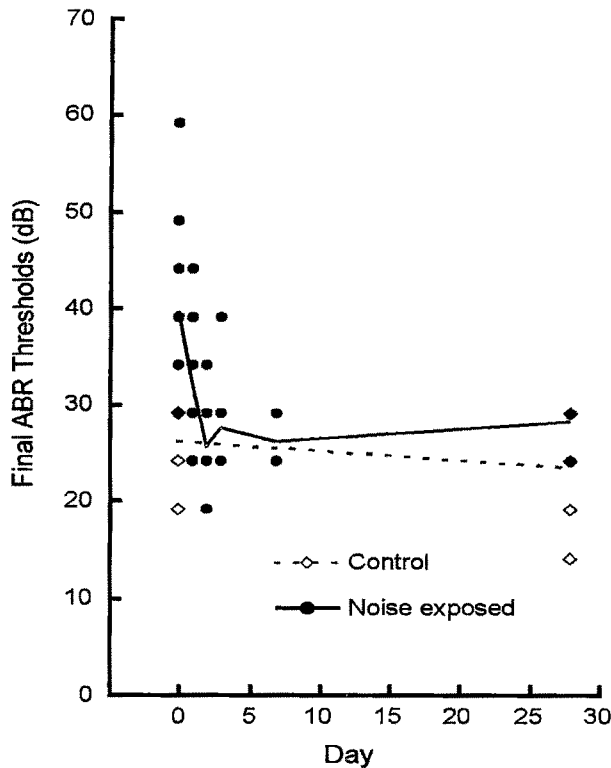


Fig 1. Final auditory brain stem response (ABR) thresholds (decibels sound pressure level) of control ears and noise-exposed ears with various survival times (days). Before third postexposure day, noise-exposed group showed significant trend of recovery of final ABR threshold ($p < .001$). From third postexposure day, trends of final ABR threshold did not significantly differ between noise-exposed group and control group ($p = .272$). Difference of final ABR thresholds between control groups was not significant throughout study period ($p = .362$).

reader (Dynatech, MR-7000, Ashford, England). Total protein (in micrograms) was determined versus a BSA standard curve. Enzyme activity was determined by measuring the amount of inorganic phosphate (Pi) released from substrate ATP colorimetrically. The lateral wall specimens of the individual cochlea were homogenized by ultrasonication (Sonics and Materials, VC-600, Danbury, Connecticut). Assays of the activities of sodium potassium magnesium adenosine triphosphatase ($\text{Na}^+, \text{K}^+, \text{Mg}^{2+}$ -ATPase) and magnesium adenosine triphosphatase (Mg^{2+} -ATPase) were initiated by adding the reaction mixture containing 354 mmol/L sodium chloride, 14.4 mmol/L potassium chloride, 3.6 mmol/L magnesium chloride, 37.5 mmol/L sodium hydrogen carbonate (NaHCO_3), 1.5 mmol/L ethyleneglycol bis(amino-ethylether) tetra-acetate (EGTA), 33.3 mmol/L glucose, and 9 mmol/L ATP, with or without 1 mmol/L ouabain, respectively. The lateral wall homogenates (10 μL) of individual cochleas were incubated for 40 minutes at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ in the reaction mixture. The OD was determined at 630 nm with the ELISA reader. Na^+, K^+ -ATPase activity was obtained by subtracting

TABLE 2. MEANS OF ENZYME ACTIVITY IN COCHLEAR LATERAL WALL OF VARIOUS NOISE-EXPOSED GROUPS AND CONTROL GROUPS

Group	n	Survival (d)	Na^+, K^+ -ATPase	Ca^{2+} -ATPase
N-1	10	0	$10.08 \pm 2.67^*$	$8.25 \pm 2.18^*$
N-2	10	1	$9.72 \pm 0.83^*$	$9.53 \pm 1.28^*$
N-3	10	2	$12.46 \pm 2.02^*$	$11.84 \pm 2.45^*$
N-4	10	3	$12.17 \pm 1.40^*$	$11.89 \pm 1.94^*$
N-5	10	7	$12.30 \pm 1.76^*$	$12.53 \pm 2.08^*$
N-6	12	28	$15.77 \pm 2.59^\dagger$	$14.04 \pm 2.23^\ddagger$
C-1	10	0	16.37 ± 3.38	16.75 ± 4.51
C-2	8	28	15.05 ± 1.92	15.71 ± 2.53

Data are mean $\pm$ SD in micromoles of inorganic phosphate per milligram of protein per hour.
All controls are pooled together as 1 control group.
* $p < .01$ versus controls (t -test).
 $^\dagger p = .99$ versus controls (t -test).
 $^\ddagger p = .07$ versus controls (t -test).

Mg^{2+} -ATPase activity from the simultaneously assayed $\text{Na}^+, \text{K}^+, \text{Mg}^{2+}$ -ATPase activity. $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity was assayed by adding 1 mmol/L ouabain and 3.1 mmol/L calcium chloride but omitting EGTA from the reaction mixture described above. Ca^{2+} -ATPase activity was calculated by subtracting Mg^{2+} -ATPase activity from $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity. The enzyme-specific activity was expressed as micromoles Pi liberated per milligram of protein per hour.

RESULTS

The ABR thresholds increased in all noise-exposed guinea pigs. There were no significant differences in initial ABR thresholds among the 8 study groups ($p = .10$, analysis of variance, $n = 80$). The difference in ABR thresholds of the same ear between the preexposure and postexposure states was statistically significant for all noise-exposed groups ($p < .01$, paired t -test). The difference in ABR thresholds of the same ear between the preexposure state and termination was statistically significant only for noise-exposed groups N-1 and N-2 ($p < .01$, paired t -test). The difference in mean ABR thresholds before and after noise exposure was 16.1 dB (95% confidence interval, 7.6 to 24.6 dB) for noise-exposed ears. The mean ABR thresholds of the different noise-exposed groups and the control groups are summarized in Table 1. The mean ABR threshold returned to the preexposure level in all groups surviving more than 2 days. Therefore, a single continuous exposure of white noise at 105 dB SPL for 10 minutes resulted in a TTS from which the ear later completely recovered. The difference in mean ABR thresholds at termination between the 2 control groups was not statistically significant ($p = .21$, t -test). The trend of hearing recovery is shown by the final ABR thresholds of noise-

TABLE 3. PERCENTAGES OF MEAN ENZYME ACTIVITIES IN COCHLEAR LATERAL WALL OF VARIOUS NOISE-EXPOSED GROUPS AS COMPARED WITH CONTROL GROUP

Group	n	Survival (d)	Na ⁺ ,K ⁺ -ATPase	Ca ²⁺ -ATPase
N-1	10	0	0.64	0.51
N-2	10	1	0.62	0.59
N-3	10	2	0.79	0.73
N-4	10	3	0.77	0.73
N-5	10	7	0.78	0.77
N-6	12	28	0.99	0.86

All controls are pooled together as 1 control group.

exposed ears with various survival times (Fig 1). Before the third postexposure day, the noise-exposed group showed a significant trend of recovery of the ABR threshold ($p < .001$). From the third postexposure day, trends of recovery for the ABR threshold did not significantly differ between the noise-exposed group and the control group ($p = .272$).

The means of Na⁺,K⁺-ATPase specific activity of

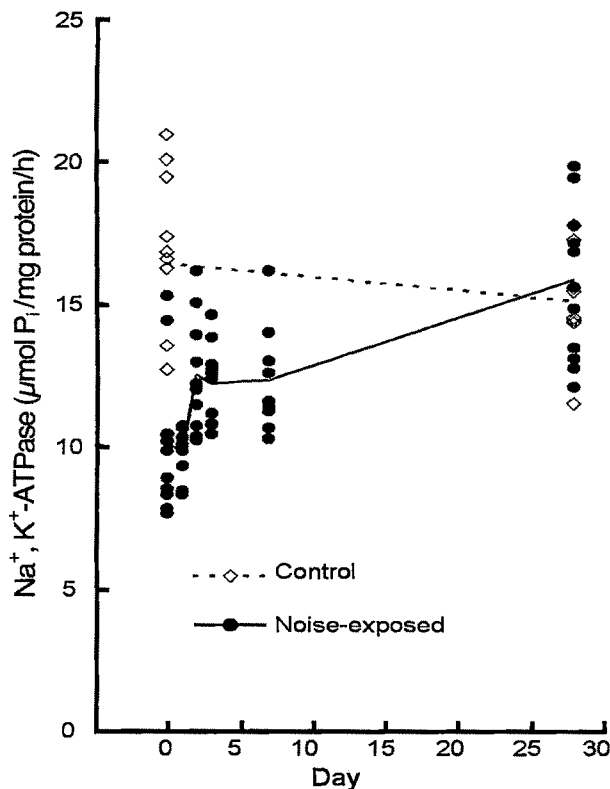


Fig 2. Specific activity (micromoles of inorganic phosphate [Pi] per milligram of protein per hour) of Na⁺,K⁺-ATPase depicted for each lateral cochlear wall across 2 control groups and 6 noise-exposed groups with various survival times (days). Before third postexposure day, noise-exposed group showed significant trend of recovery in specific activity of Na⁺,K⁺-ATPase ($p = .025$). Difference in specific activity of Na⁺,K⁺-ATPase between 2 control groups was not significant throughout study period ($p = .22$).

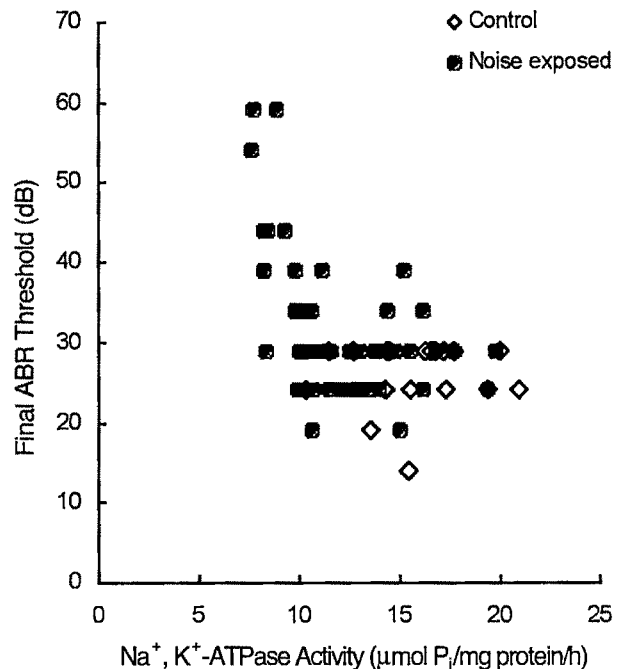


Fig 3. Association between final ABR threshold and specific activity of Na⁺,K⁺-ATPase. Pearson's correlation coefficient was significant for Na⁺,K⁺-ATPase (-0.419 , $p = .001$) in noise-exposed group. Same coefficient was not significant in control group.

the various noise-exposed groups and the control groups are summarized in Table 2. The relative mean specific activities of Na⁺,K⁺-ATPase in the cochlear lateral wall of various noise-exposed groups as compared with the control groups are shown in Table 3. Na⁺,K⁺-ATPase specific activities in the cochlear lateral wall showed no significant difference between the 2 control groups with a 28-day difference of survival time ($p = .34$, t -test). A significant decrease of Na⁺,K⁺-ATPase specific activity was noticed in all noise-exposed groups except for group N-6 ($p < .01$, t -test). The distribution of Na⁺,K⁺-ATPase specific activities in the cochlear lateral wall of control ears and noise-exposed ears after different survival times is displayed in Fig 2. The association between the final ABR threshold and specific activity of Na⁺,K⁺-ATPase in the cochlear lateral wall is shown in Fig 3. There was a significant negative association between the final ABR threshold and Na⁺,K⁺-ATPase specific activity.

The means of Ca²⁺-ATPase specific activity of the various noise-exposed groups and the control groups are summarized in Table 2. The relative mean specific activities of Ca²⁺-ATPase in the cochlear lateral wall of various noise-exposed groups as compared with control groups are shown in Table 3. The Ca²⁺-ATPase specific activities in the cochlear lateral wall showed no significant difference between the 2 control groups with a 28-day difference of sur-

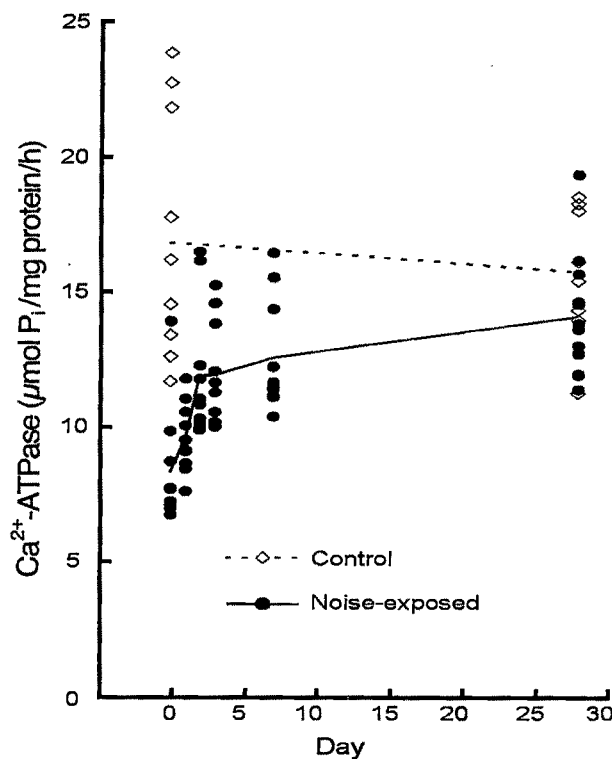


Fig 4. Specific activity (micromoles of inorganic phosphate per milligram of protein per hour) of Ca^{2+} -ATPase depicted for each lateral cochlear wall across 2 control groups and 6 noise-exposed groups with various survival times (days). Before third postexposure day, noise-exposed group showed significant trend of recovery for specific activity of Ca^{2+} -ATPase ($p = .001$). Difference for specific activity of Ca^{2+} -ATPase between 2 control groups was not significant throughout study period ($p = .39$).

vival time ($p = .57$, t -test). A significant decrease in Ca^{2+} -ATPase specific activity was noted in all noise-exposed groups except for group N-6 ($p < .01$, t -test). The distribution of Ca^{2+} -ATPase specific activities in the cochlear lateral wall of control ears and noise-exposed ears for different survival times is displayed in Fig 4. The association between the final ABR threshold and specific activity of Ca^{2+} -ATPase in the cochlear lateral wall is shown in Fig 5. There was a significant negative association between the final ABR threshold and Ca^{2+} -ATPase specific activity.

DISCUSSION

Noise-induced TTS seems to be related to reversible biochemical or biophysical processes in the cochlea. In the present study, statistical analysis indicated that a good correlation existed between the recovery of hearing function and the specific activities of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall of noise-exposed ears with TTS. Complete recovery of the ABR threshold was noted in all noise-exposed groups with a survival time of 2 days or more. In 81% of these noise-exposed ears

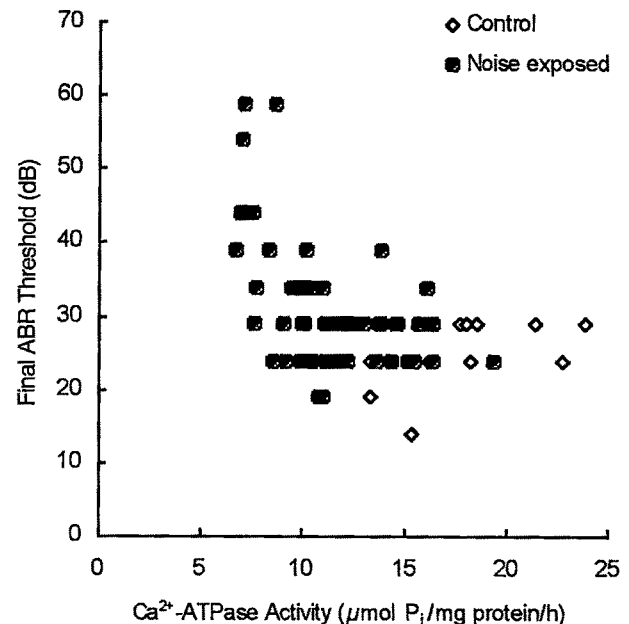


Fig 5. Association between final ABR threshold and specific activity of Ca^{2+} -ATPase. Pearson's correlation coefficient was significant for Ca^{2+} -ATPase (-0.475 , $p < .001$) in noise-exposed group. Same coefficient was not significant in control group.

(34/42), Na^+ , K^+ -ATPase specific activity in the cochlear lateral wall was at least 70% of the mean specific activity of the control ear. In 70% of these noise-exposed ears (29/42), Ca^{2+} -ATPase specific activity in the cochlear lateral wall was at least 70% of the mean specific activity of the control ear. In contrast, in noise-exposed ears with a permanent threshold shift, the specific activities of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall were only 27% to 48% and 28% to 47% those of the control ears, respectively.²⁹ The present findings suggest that the levels of specific activities of Na^+ , K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall required to maintain a normal ABR are approximately 70% of the normal level. It may be true that higher levels of these enzyme activities are required for all functions of the living cochlea.

Disruption of the mechanoelectrical transduction process at the apex of the outer hair cells is one of the mechanisms suggested for noise-induced TTS. Disruption of the mechanoelectrical transduction process is presumed to inactivate the opening of the mechanoelectrical transduction channels, resulting in a reduction of hair cell receptor currents that is related to a decrease in neural sensitivity in a quantitatively similar way.⁷ Disruptions producing threshold elevation can result from short-lived morphological changes in the tip links of hair cell stereocilia,^{7,10,11} a molecular inactivation of the mechanoelectrical transduction channels,^{14,15} or other mechanical and morphological damage to the stereocilia and hair

cells.^{6,8,9,13}

There are some discrepancies in the literature as to stereociliary abnormalities underlying noise-induced TTS. The disagreement probably arises from the different histopathologic changes noted during the different phases after noise exposure. Liberman and Dodds⁶ and Gao et al⁸ provided evidence for a correlation between morphological changes of stereocilia and temporary hearing loss. The extent of stereociliary changes seemed to be further reduced and the threshold shift was less than 10 dB after a survival time of 48 hours. A regular arrangement of stereocilia on hair cells was observed, and no additional threshold shift was shown 4 days after exposure. In the present study, TTS was induced by similar broadband white noise, and the recovery process of quantitative enzyme activities also coincided with that of both auditory function and morphological damage in the hair cell stereocilia, as likewise observed by Liberman and Dodds and Gao et al.

Current evidence shows a substantial temporal disparity between the appearance and maturation of new hair cells and the recovery of peripheral auditory function. The multiexponential nature of the onset and recovery of TTS suggested a simple molecular explanation for the closure of the mechanoelectrical transduction channels.^{14,15} It was proposed that at least one component of TTS is due to a reversible molecular inactivation of the mechanoelectrical transduction channels, rather than to a morphological disruption of the stereocilia, tip links, or other cochlear structures. Other researchers expected destruction of the tip link integrity of stereocilia to cause disruption of mechanoelectrical transduction and a concomitant hearing loss in TTS. The correlation between endolymphatic Ca^{2+} and hair cell tip links has been shown previously. Destruction of hair cell tip links by exposure to a Ca^{2+} chelator resulted in a significant reduction in hair cell receptor current, as the transduction channels were presumably rendered inoperative.^{11,19} An almost complete destruction of the tip links was verified, and relative stereocilia motion was altered in chick cochlear hair cells after exposure to culture medium containing low Ca^{2+} levels.³⁰ It was also speculated that the recovery of normal hearing was ultimately limited by tip link regeneration in noise-induced TTS. Therefore, inner ear Ca^{2+} is important for the mechanoelectrical transductive capacity of cochlear hair cells. Reduction of Ca^{2+} -ATPase specific activity in the cochlear lateral wall may imply a decrease in endolymphatic Ca^{2+} and disruption of mechanoelectrical transduction. In addition, the sensory transduction mechanism has also been shown to be driven by a standing current that is mainly driven by K^{+} .^{31,32} Reduction of Na^{+} , K^{+} -ATPase specific

activity in the cochlear lateral wall may imply a decrease of K^{+} influx to the endolymph, a reduction in the standing K^{+} influx through the hair cells, and disturbance of sensory transduction. Recovery of specific activities of Na^{+} , K^{+} -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall may imply recovery of endolymphatic Ca^{2+} and K^{+} influx, resulting in normal transduction processes of hair cells. According to the present results, we suggest that the reduction and recovery of specific activities of Na^{+} , K^{+} -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall be implicated in the alterations of noise-induced TTS.

In the present study, a significant trend of recovery for specific activities of Na^{+} , K^{+} -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall was shown before the third postexposure day. In addition, it is clear that Na^{+} , K^{+} -ATPase and Ca^{2+} -ATPase specific activity levels are better fitted by a curvilinear rather than a linear function (Figs 3 and 5). As an alternative to reversible inactivation of mechanoelectrical transduction channels, it may be proposed that reversible inactivation of Na^{+} , K^{+} -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall is another component of TTS. The possibility exists that the reduction of Na^{+} , K^{+} -ATPase or Ca^{2+} -ATPase specific activities in the cochlear lateral wall is the result of reduced stria blood supply due to acoustic trauma. Nakai and Masutani⁴ showed that sound exposure resulted in vasoconstriction of the blood vessels of the cochlear lateral wall and spiral lamina. Sludging of the blood cells was also recognized in these blood vessels. They also found that the above phenomenon was reversible and inferred that the cause of the noise-induced threshold shift lay in impaired blood flow due to vasoconstriction of the cochlear capillary. Rarey et al³³ showed that a reduced stria blood supply could reduce hormonal levels, resulting in a significant reversible reduction in Na^{+} , K^{+} -ATPase activity. According to the above studies, the maintenance of normal auditory function could be considered to be related to the stria blood supply and specific enzyme activities in the cochlear lateral wall in a quantitatively similar way. Although there is no proof or indication based on the present material that changes in the stria blood supply have occurred as a result of the noise stimulation, we speculate that an altered stria blood supply may play a role in the reduction and recovery of the specific activities of Na^{+} , K^{+} -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall in noise-induced TTS.

Reductions in the blood supply to the stria vascularis can depress the endocochlear potential and, in turn, affect all other cochlear potentials. Gratton et al³⁴ showed a correlation between the magnitude of the endocochlear potential and the total area of the

stria that remained vascularized. They observed that at least 80% of the stria was found to contain capillaries of normal density and diameter in aged gerbils with a normal or near-normal endocochlear potential magnitude. Schulte and Schmiedt³⁵ investigated the correlation between the volume of Na⁺,K⁺-ATPase immunoreactivity in the stria vascularis and the magnitude of the endocochlear potential for quiet-aged gerbils. They showed that an endocochlear potential of near-normal magnitude could be measured only in ears retaining Na⁺,K⁺-ATPase immunoreactivity in at least 80% of the stria for quiet-aged gerbils. Interestingly, the present study showed a quantitatively similar finding that a critical level (70% of the control) of Na⁺,K⁺-ATPase and Ca²⁺-ATPase specific activities was closely related to the complete recovery of ABR thresholds in guinea pigs with noise-induced TTS.

In conclusion, the present findings confirm the role

of both Ca²⁺-ATPase and Na⁺,K⁺-ATPase in the recovery of auditory function in noise-induced TTS. Although other factors may be involved, restoration of Na⁺,K⁺-ATPase and Ca²⁺-ATPase specific activities to a critical level is closely related to the complete recovery of auditory function in noise-induced TTS. To the best of our knowledge, there are no reported data in the literature concerning the changes of the endocochlear potentials and endolymphatic Ca²⁺ levels induced by the same noise exposure paradigm as that used in the present study. Further studies are required to support our present hypothesis. The present study only assayed the specific activities of Na⁺,K⁺-ATPase and Ca²⁺-ATPase, and did not measure the lateral wall protein levels of these enzymes. However, there is a possibility that the lateral wall protein levels of these enzymes were down-regulated in response to noise trauma. Our present findings could drive further studies on the molecular basis of noise-induced hearing loss.

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CLINICAL NOTE

MENINGIOMA INVOLVING MECKEL'S CAVE: TRANSPETROSAL SURGICAL ANATOMY AND CLINICAL CONSIDERATIONS

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Meningiomas originating in Meckel's cave (MC) are uncommon lesions that represent 1% of all intracranial meningiomas. Innovations in skull base surgery have enabled resection of these lesions with less morbidity, but require an intimate knowledge of both lesional pathology and regional microneuroanatomy. To review the surgical and clinical considerations involved in the management of MC meningiomas, we retrospectively reviewed data from patients who underwent transpetrosal resection of primary MC meningiomas between 1984 and 1998. Of 146 patients who underwent transpetrosal removal of meningiomas, 7 were believed to have tumors originating in MC. All 7 patients presented with trigeminal dysfunction, facial pain, and/or headache. Complete tumor removal was achieved in 5 of the 7 patients. Facial hypoesthesia or anesthesia, paralysis of cranial nerve VI, and ophthalmoplegia were among the postoperative complications encountered. Meningiomas of MC represent treatable lesions whose diagnosis requires prompt imaging of patients with trigeminal dysfunction and symptoms of facial pain and headache.

KEY WORDS — Meckel's cave, meningioma, skull base, transpetrosal approach, trigeminal nerve.

INTRODUCTION

Primary Meckel's cave (MC) meningiomas are uncommon tumors representing about 1% of all intracranial meningiomas.¹ Given the rarity of these tumors and the expanding repertoire of skull base approaches available to surgically manage these lesions, no clear consensus has been reached regarding the management of MC meningiomas. In an attempt to recognize characteristics and generalize treatment of patients with MC meningiomas, earlier reports had divided patients into 2 groups on the basis of whether the lesion was confined to MC (group I) or extended beyond MC (group II).¹ However, subsequent clinical investigations pointed to the diversity of patients and tumors within each of these 2 groups and the different surgical techniques indicated by the various disorders.² As a result, these authors further subdivided MC meningiomas into tumors confined to MC (group 1), tumors originating in MC and extending into the middle fossa (group 2), tumors originating in MC and extending into the posterior fossa (group 3), and tumors originating in MC and involving both middle and posterior fossae (group 4).² This classification system reflects the nature of these lesions, as well as the anatomic confines and potential spaces related to MC that determine how these tumors expand out of MC. Despite these helpful reports, further data are needed to expand the cumula-

tive experience in the literature with regard to management of patients with MC meningiomas.

Therefore, we reviewed our experience with transpetrosal surgery, specifically in patients with MC meningiomas. Our data consist of clinical information from patients with pathologically confirmed meningiomas restricted to MC, as well as those extending beyond the confines of MC to involve the cavernous sinus and/or the middle and posterior fossae. Although classification schemes for these lesions can provide a useful framework for discussing their characteristics, we find that the surgical approach and management of the individual tumors is dictated by the precise location, route of extension, and preoperative cranial nerve (CN) function and deficits. This report describes the clinical features of our patients with MC meningiomas (which are consistent with those of earlier reports²⁻⁴), their presenting symptoms, our surgical technique and outcomes, and a review of the pertinent microneuroanatomy of MC.

MATERIALS AND METHODS

In this retrospective study, 7 patients (5 women and 2 men; mean age, 34.6 years; range, 19 to 52 years) with primary MC meningioma were identified out of 146 patients who underwent transpetrosal removal of meningiomas at our institution between 1984 and

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1998. From this overall group, 53 patients had undergone anterior petrosectomy, 38 posterior petrosectomy, and 55 combined anterior-posterior petrosectomy. The lesions were identified as true MC meningiomas on the basis of intraoperative observations with pathological confirmation. All 7 patients with primary MC meningiomas presented with symptoms of facial pain and/or headache at the initial evaluation and demonstrated deficits of CN V on physical examination.

Clinical data (eg, presenting histories, symptoms, physical examination findings) were collected from neurologic and neurosurgical medical records, as were preoperative and postoperative imaging data, as well as audiological and intraoperative data. These data were tabulated, collated, and analyzed with respect to patient demographics, tumor characteristics, surgical technique used for resection, and 2-year follow-up data.

Surgical Technique. Our technique has been previously described^{5,6} and, therefore, is only briefly summarized here. The patient is placed in the lateral supine oblique position with the head fixed in a Mayfield 3-point fixation headholder. The head is turned 60° and flexed. An armored endotracheal tube helps to avoid airway compromise.

A preauricular curvilinear skin incision is carried above the auricle and directed in a C-shaped fashion behind the auricle. If needed, an anterior limb may be directed in the hairline to allow for exposure of the frontozygomatic region in cases of cavernous sinus involvement.

Two pairs of bur holes straddle the sigmoid-transverse junction and the transverse sinus. When connected, this series of bur holes allows simultaneous exposure of both the temporal and suboccipital regions. Excess bone is drilled to reduce the temporal bone flush with the floor of the middle fossa laterally and to expose the sigmoid sinus inferiorly. Anterior or posterior petrosectomy or both may be performed.

Cortical mastoidectomy is performed. The posterior canal wall is thinned, and the central mastoid air cell tract is followed along the tegmen plate to the aditus ad antrum. The horizontal canal and lateral process of the incus are noted. The facial nerve is identified in its vertical segment, and retrofacial cells are opened. The sigmoid sinus is skeletonized to the level of the jugular bulb. Superiorly, the sinodural angle is opened widely and drilled medially to the level of the superior petrosal sinus. Perilabyrinthine cells are opened, and the posterior and superior canals are skeletonized. The dura mater over the poste-

rior fossa, endolymphatic sac, and floor of the middle fossa is then exposed.

From a subtemporal (middle fossa) exposure, the greater superficial petrosal nerve is followed to the geniculate ganglion, and the internal auditory canal is skeletonized. Anteriorly, the horizontal petrous carotid artery is identified, and Kawase's triangle is widely opened. At the juncture of the vertical and horizontal parts of the petrous carotid artery, extreme care is taken to avoid violating the cochlea.

The dura mater is opened infratentorially cephalic to the dome of the jugular bulb, and the incision is extended in a superior direction, avoiding the endolymphatic duct. Superiorly, the incision is carried along the floor of the temporal fossa, and medially, it is directed superior to the transverse sinus. The superior petrosal sinus is sacrificed with gentle retraction to preserve the vein of Labbé.

The sigmoid sinus, vein of Labbé, and cerebellum are gently retracted in a posterior direction, and the temporal lobe is gently lifted upward. Care is taken to avoid excessive cerebellar, temporal lobe, and venous retraction (Labbé).

In cases of supratentorial extension, the tentorium is incised to the level of the tentorial notch. With this opening, the upper pole and anterolateral extent of the lesion may be well exposed.

A watertight closure is accomplished by using fascia lata and fat. The air cells are waxed, and a piece of muscle is placed in the epitympanum. Microplating systems have been successful in replacing the craniotomy bone.

RESULTS

Patient Characteristics. The gender distribution of the 7 patients with MC meningioma was consistent with that in other reports on this disease process.¹ The presenting symptoms of facial pain and/or headache at the time of initial evaluation and the deficits of CN V found on physical examination were in contrast to other reports, in which more than 30% of patients with MC meningiomas had negative neurologic examinations.¹ No particular division of CN V was more affected than any other in this small cohort of patients. Other CN involvement (CNs III, IV, VI, VII, and VIII) was noted in 4 patients (Table 1).

Tumor Characteristics and Surgical Outcomes. Meningiomas ranged in size from 1.5 to 4.5 cm (mean, 2.8 cm), according to preoperative magnetic resonance imaging (MRI) data and surgical observations. Using surgical approaches tailored to the individual tumor size and location (Table 2), we accomplished

TABLE 1. CLINICAL SYMPTOMS AND FINDINGS AT TIME OF DIAGNOSIS

Case	Age (y)	Sex	CN V Deficit	Other CN Deficits	Facial Pain	Headache	Seizure	Cavernous Sinus Involved	Posterior Fossa Involved
1	23	F	V2-V3	III	+	+		+	
2	46	M	V1-V3		+	+			
3	52	M	V1-V3	VI, VII, VIII	+	+	+		+
4	41	F	V3	III, IV		+		+	
5	19	F	V1-V2	VI, VIII	+				+
6	28	F	V1-V3		+	+			+
7	33	F	V1-V3		+				+

CN — cranial nerve.

total tumor removal in 5 cases, and attained subtotal tumor removal in the remaining 2 cases. One of these patients, who had a particularly aggressive vascular tumor and had undergone subtotal tumor resection, subsequently underwent postoperative radiotherapy. At a minimum of 2 years of follow-up, MRI data showed no evidence of disease in 5 patients and no growth of the residual tumor in the 2 patients who underwent subtotal tumor removal. The long-term postoperative sequelae included facial hypoesthesia or anesthesia in 4 patients, CN VI paralysis in 2 patients, ophthalmoplegia in 1 patient, and sensorineural hearing loss with tinnitus in another patient. No major complications (death, hydrocephalus, seizure, cerebrospinal fluid leakage, or meningitis) were noted in this series of 7 patients.

DISCUSSION

Clinical Considerations. The primary symptoms and signs that triggered diagnostic imaging in these patients were facial pain, headache, and deficits of one or more trigeminal divisions. Prior investigators had attempted to correlate various tumor sizes and locations with specific aspects of trigeminal neuralgia versus atypical facial pain.^{3,7,8} However, no such correlation could be found in our patient series. The

impairment of cranial nerves other than CN V was fairly common in this group of patients, as well. Given the proximity and anatomic relationship of MC to the cavernous sinus (discussed below), it is not surprising that nerves of the cavernous sinus were (relatively) frequently involved (cases 1, 3, 4, and 5). One patient, with a 4.0-cm-diameter mass involving both middle and posterior fossae, showed deficits of CNs VII and VIII, demonstrating the need for appropriate audiovestibular and facial nerve evaluation in patients with MC meningiomas. In contrast to earlier reports, all of our patients experienced some sort of CN deficit preoperatively. This finding differs from the data of Delfini et al,¹ in which more than 30% of the patients had normal profiles on neurologic examinations. We believe that all patients with facial pain (with or without persistent headache) and any focal deficits on CN examination should undergo diagnostic imaging (Fig 1). Further workup should include audiovestibular testing and evaluation of facial nerve function as indicated. After initial gadolinium-enhanced MRI studies, several authors have advocated carotid occlusion studies when significant carotid artery involvement is noted on scans.

Surgical Anatomy. An appreciation of the intricate anatomy of MC is required not only for understand-

TABLE 2. TUMOR SIZE, SURGICAL MANAGEMENT, AND TWO-YEAR OUTCOME

Case	Tumor Diameter (cm)	Surgical Approach	Surgical Result	Postoperative CN Deficits	Postoperative MRI (minimum 2 y)
1	3.5	Dolenc–anterior petrosectomy	Total removal	CN VII hypoesthesia	NED
2	1.5	Subtemporal–anterior petrosectomy	Total removal	None	NED
3	4.0	Transtemporal, suboccipital, anterior-posterior petrosectomy	Subtotal removal	Facial anesthesia, CN VI paralysis, SNHL, tinnitus	No growth*
4	2.0	Dolenc–anterior petrosectomy	Total removal	CN VII hypoesthesia, ophthalmoplegia	NED
5	4.5	Transtemporal, suboccipital, anterior-posterior petrosectomy	Total removal	CN VII hypoesthesia, CN VI paralysis	NED
6	2.5	Subtemporal–anterior petrosectomy	Subtotal removal	None	No growth
7	2.0	Subtemporal–anterior petrosectomy	Total removal	None	NED

MRI — magnetic resonance imaging, NED — no evidence of disease, SNHL — sensorineural hearing loss.

*Patient also underwent postoperative radiotherapy.



Fig 1. T1-weighted gadolinium-enhanced magnetic resonance image of right Meckel's cave meningioma. Note bright homogeneous enhancement of lesion with small limbs of enhancement along dura extending anteriorly (toward clivus) and posteriorly.

ing the surgical approach to lesions of the space, but also for understanding the behavior and routes of extension of tumors involving MC.

The inferior (ventral) aspect of MC demonstrates the foramina transmitting the 3 divisions of CN V

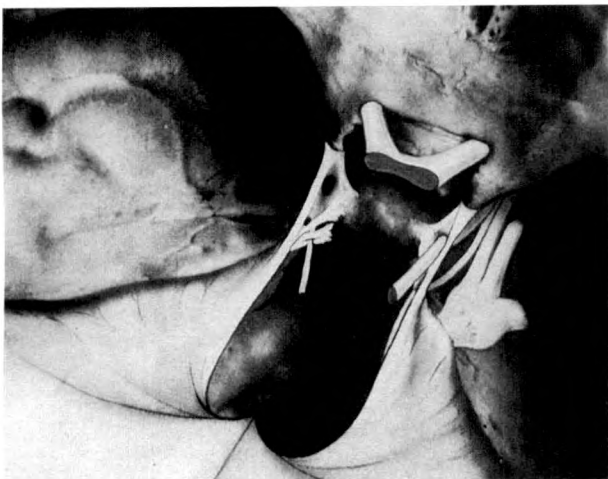


Fig 2. Drawing of Meckel's cave region as viewed from above. Right side demonstrates trigeminal ganglion with exiting of 3 divisions through floor of middle fossa. Left side shows prominent foramen ovale with semilunar ganglion removed. Proximity of cranial nerves III, IV, and VI to Meckel's cave as they course through cavernous sinus is clearly shown. (Reprinted with permission from Mayfield Clinic, Cincinnati, Ohio.)

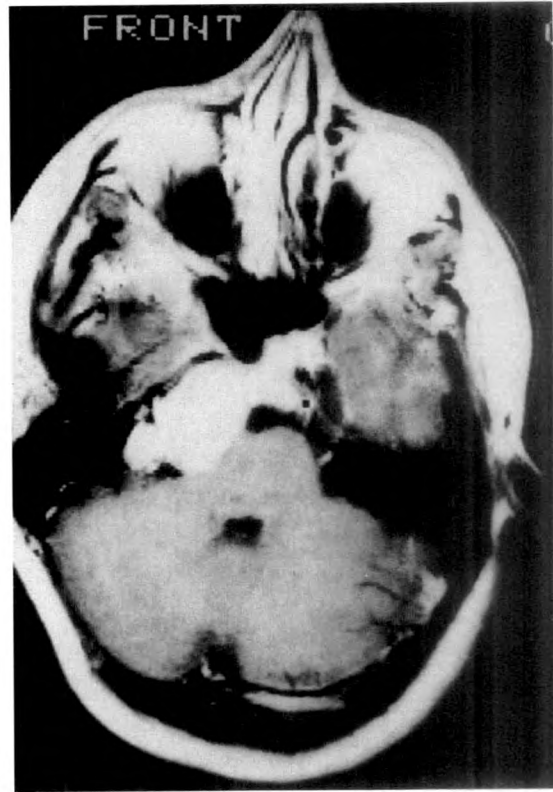


Fig 3. T1-weighted gadolinium-enhanced magnetic resonance image of large right Meckel's cave meningioma involving posterior and middle fossae and clivus and crossing midline. Note brain stem compression and apparent extension into sphenoid sinus.

(Fig 2). Laterally, dural folds delineate MC from the petrous apex, whereas superior dural folds (forming the roof of MC) become continuous with the lateral wall of the cavernous sinus anteriorly and the tentorium more posteriorly. Also related superior-medial to the roof of MC is Dorello's canal, transmitting CN VI.⁴ Significantly, the intrapetrous carotid artery also runs posterolateral to MC and is again in close proximity as it transitions to the intracavernous portion below the trigeminal ganglion.

The medial abutment of MC to the cavernous sinus explains the proclivity of lesions to involve this region and the associated ocular CNs. The continuity of the posterior-superior dural folds covering MC with the tentorium explains how tumors originating in MC traverse the petrous bone to involve the posterior fossa and give rise to the typical dumbbell-shaped lesions. As lesions extend further, all dural surfaces in contact with MC (eg, clivus) can become infiltrated with tumor and make total resection more difficult. Involvement of multiple dural surfaces is fairly typical and may include all 5 surfaces: petrous, clivus, MC, tentorium, and cavernous sinus⁹ (Fig 3).

Our surgical approach to meningiomas of MC is individualized and determined by the extent of mid-

dle and posterior fossa involvement as demonstrated by preoperative MRI. Meningiomas limited to MC or primarily middle fossa in involvement are approached through a subtemporal-transpetrosal technique with division of the tentorium if access is required for a small posterior fossa component. For tumors with involvement of both the middle and posterior fossae, a combination of subtemporal, transpetrosal, and suboccipital approaches has been used (eg, cases 3 and 5). Such wide exposure facilitated total tumor removal in case 5, which was, incidentally, the largest lesion in this series (4.5 cm).

Interestingly, a recent report by Samii et al¹⁰ proposed an alternative retrosigmoid-intradural-suprameatal approach to MC that achieved improved exposure and avoidance of large petrous bone resections. The authors suggested that such a technique reduced the risks of hearing loss, facial palsy, and cerebrospinal fluid leakage as compared with current techniques that involve subtemporal approaches with various degrees of petrosectomy and tentorium division. Our initial experience with patients with these tumors sug-

gests that our subtemporal-transpetrosal approach to MC meningiomas is a relatively safe operation with relatively low morbidity. Significantly, no major complications (death, hydrocephalus, seizures, cerebrospinal fluid leakage, or meningitis) were encountered in this series of patients. Furthermore, 3 of the 7 patients demonstrated no CN deficits after complete tumor removal (cases 2, 6, and 7).

CONCLUSIONS

These observations suggest that these techniques of (transpetrosal) skull base surgery provide a relatively safe surgical option for management of MC meningiomas with a high rate of complete tumor resection. As described above, the intricate microanatomy is central to the behavior of these lesions, as well as to the surgical management. This report describes our experience with these rare tumors and highlights the clinical and surgical features of MC meningiomas. These data add to the growing body of literature that suggests that patients with such meningiomas can be successfully treated with contemporary skull base techniques.

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CLINICAL NOTE

ABRUPT ONSET OF SENSORINEURAL HEARING LOSS AND TINNITUS
IN A PATIENT WITH CAPILLARY TELANGIECTASIA OF THE PONS

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Capillary telangiectasia is often found incidentally on magnetic resonance imaging and may be associated with minor neurologic symptoms, but there has been little evidence about whether such lesions are responsible for these symptoms. Here we report a case of capillary telangiectasia of the right mid pons, which was associated with the abrupt onset of tinnitus and sensorineural hearing loss. Examination of the auditory brain stem responses showed abnormalities of waves III and IV on the left and bilateral prolongation of the interwave intervals. Optokinetic nystagmus showed slight left-right asymmetry.

KEY WORDS — auditory brain stem response, capillary telangiectasia, hearing loss, optokinetic nystagmus, tinnitus.

Capillary telangiectasia is a vascular malformation that is usually found incidentally at autopsy. However, magnetic resonance imaging (MRI) has greatly increased the diagnosis of capillary telangiectasia, and minor neurologic symptoms have frequently been noted in these patients.^{1,2} Although otorhinolaryngological symptoms such as hearing loss, tinnitus, vertigo, and dizziness have also been found, the otorhinolaryngological investigation of these symptoms has not been reported.

We encountered a case of capillary telangiectasia associated with the abrupt onset of hearing loss and tinnitus, and examined the audiological and vestibular function of this patient.

CASE REPORT

A 36-year-old man presented to our clinic complaining of the abrupt onset of right-sided tinnitus that had persisted for 1 week. The patient denied any vertigo or imbalance. A benign cerebral vascular tumor had been detected at another hospital 10 years earlier, but he did not know the exact diagnosis. He also had a history of otitis media, nasal allergy, anemia, urinary stone, and fracture of his third toe.

The findings on otoscopic examination were normal. Audiometry revealed slight sensorineural hearing loss on the right side (average of 35.0 dB at 500 Hz, 1,000 Hz, and 2,000 Hz; Fig 1) and also on the

left side (Fig 2). His speech discrimination was 100% on the right and left for the Japan Audiological Society 67S typed phonetically balanced word list presented at 40 dB above the speech reception threshold. Impedance testing indicated a normal pressure and compliance bilaterally. Bekesy audiometry gave a type I pattern. The minimum intensity in tone decay tests was 0 dB. The short increment sensitivity

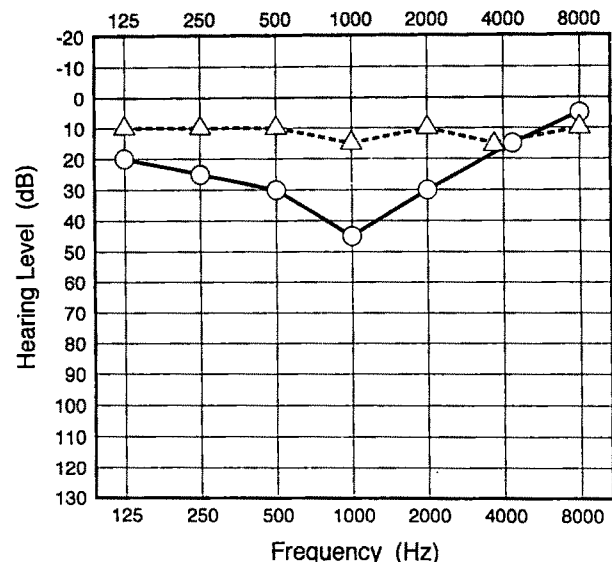


Fig 1. Pure tone audiogram shows air conduction threshold of right ear at first visit to our clinic (circles) and after 3 days of treatment (triangles). Slight sensorineural hearing loss was improved after treatment.

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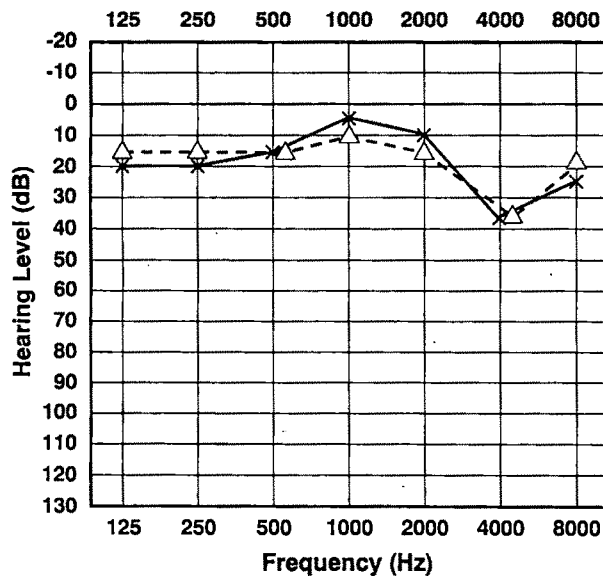


Fig 2. Pure tone audiogram shows air conduction threshold of left ear at first visit to our clinic (crosses) and 50 days later (triangles). Hearing loss of 35 dB at 4,000 Hz was persistent.

index was 95% at 1,000 Hz and 70% at 4,000 Hz on the right side versus 5% at 1,000 Hz and 70% at 4,000 Hz on the left side. Auditory brain stem responses (ABRs) were recorded (90 dB sensation level click signals with a sine wave of 3,000 Hz; stimulus interval of 54 ms, computer averaging of 2,000 times; ER-02, RION, Tokyo, Japan), and the amplitude of waves III and IV was smaller on the left side than on the right. In addition, the shape of wave IV was poorer on the left than on the right, and this wave was nearly absent on the left. Furthermore, there was prolongation between waves I and III, as well as waves I and V, on both sides, but there were no differences in latency or intervals between the right and left sides (Fig 3). The tinnitus affecting the right ear was equivalent in pitch to a pure tone of 1,000 Hz, and the intensity was 50 dB.

Plain X-ray films and computed tomography (CT) revealed no abnormalities of the inner ear, internal auditory canal, or posterior fossa. On contrast T1-weighted MRI, there was a region of stippled enhancement (12 mm × 10 mm × 11 mm) in the right mid pons (Fig 4A,B). A draining vein was not detected. On noncontrast T1-weighted and T2-weighted MRI, this lesion was invisible (Fig 4C,D). The pons was not enlarged, and there was no edema or hemosiderin rim around the lesion.

The results of vestibular function tests, such as the stepping test, tests of the righting reflex (including the Romberg and Mann tests), gaze nystagmus, spontaneous nystagmus, positional and positioning nystagmus, and the eye tracking test, were all within normal limits. However, optokinetic nystagmus showed

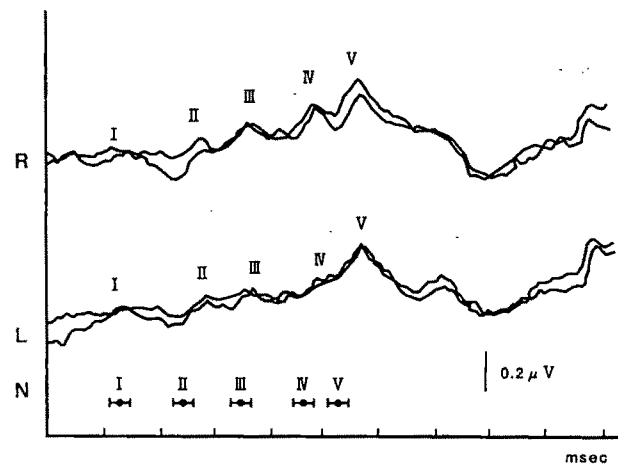


Fig 3. In auditory brain stem responses, amplitude of waves III and IV is smaller on left (L) than on right (R). In addition, shape of wave IV is poorer on left than on right, and this wave is nearly absent. There is prolongation of interval between waves I and III, as well as waves I and V, on both sides in comparison with normal control ears (N). Means $\pm$ SEM of latency in 10 normal ears are shown at bottom of graph.

slight left-right asymmetry (Fig 5). A caloric test was not done, because it was considered likely to place a load or stress on the pontine vascular lesion. The Schellong test also gave a normal result.

Hydrocortisone, adenosine triphosphate sodium, and mecobalamine were administered. An antihistamine was also administered for associated nasal allergy. His hearing improved on the right after 3 days of treatment (Fig 1), and the tinnitus resolved after 2 weeks. However, a sensorineural hearing loss (35 dB at 4,000 Hz) persisted on the left after 50 days (Fig 2).

DISCUSSION

Capillary telangiectasias are usually lesions less than 2 cm in diameter, and are most frequently located in the pons.³ Their estimated prevalence in autopsy series is 0.7%.⁴ Microscopic examination shows that these lesions are composed of numerous dilated capillaries running among normal tissue elements.⁵

We diagnosed this case as capillary telangiectasia on the basis of the characteristic MRI appearance and the benign clinical course. On MRI, these lesions show slight contrast enhancement (homogeneous, irregular brushlike border, or stippled) and marked gradient-echo signal loss, but may be otherwise undetectable on T1- and T2-weighted images, except for the possible existence of a draining vein.

Cavernous angioma is also frequently found in the brain stem, but has different features on T2-weighted MRI (a surrounding hypointense hemosiderin ring with an internal "popcorn" appearance).^{1,2} Mixed or

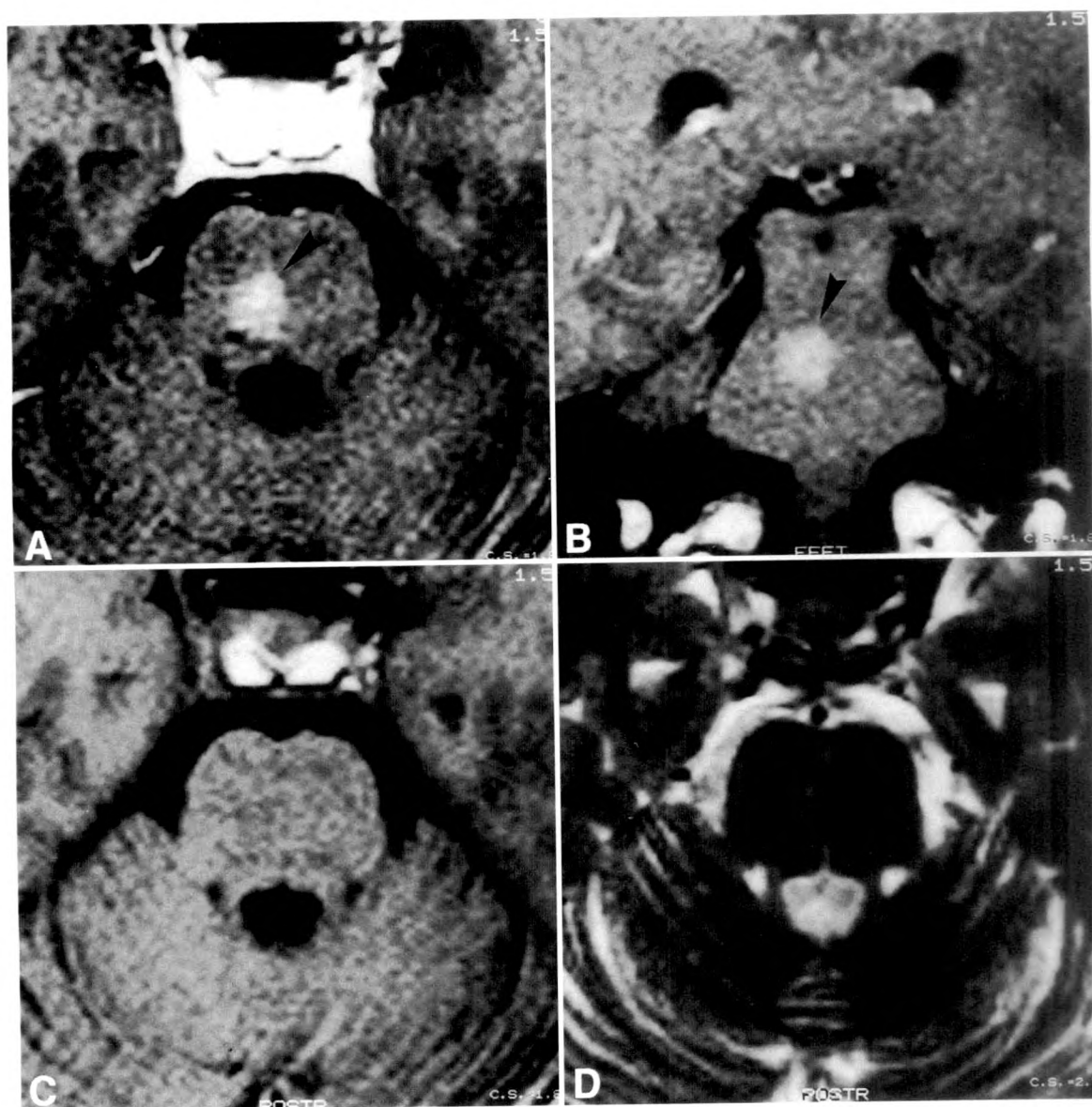


Fig 4. Axial (A) and coronal (B) contrast-enhanced T1-weighted magnetic resonance images show stippled enhancement in right mid pons (arrowheads). Plain axial T1-weighted image (C) and axial T2-weighted image (D) are normal.

transitional forms of cerebral vascular malformations have also been reported, and the symptoms and signs of such lesions are more complex.¹

The differential diagnosis of brain lesions that show enhancement on MRI includes neoplasia, subacute infarction, demyelination, and inflammation, and gradient-echo imaging is a useful technique for diagnosing capillary telangiectasia.²

Patients with capillary telangiectasia usually show a benign clinical course and may be asymptomatic or may present with mild symptoms such as headache, vertigo, dizziness, tinnitus, hearing loss, mild facial weakness, and gait disturbance.^{1,2} These lesions are often discovered incidentally.² However, there have been reports of hemorrhage,^{6,7} as well as

of aggressive and progressive lesions associated with progressive neurologic deterioration that resulted in death.⁸ Barr et al¹ and Lee et al² investigated the frequency of posterior fossa symptoms in these patients, including tinnitus, hearing loss, vertigo, and dizziness (see Table^{1,2,6,8-10}), but the results of audiological and vestibular function tests have not been reported previously.

Our patient complained of the abrupt onset of right-sided tinnitus, and audiometry revealed slight sensorineural hearing loss on the right that improved soon after the start of treatment (Fig 1). The average pre-treatment hearing loss (at 1 week after the onset) was 35 dB. However, the left-sided sensorineural hearing loss of 35 dB at 4,000 Hz did not change throughout the treatment or follow-up (Fig 2). Our patient

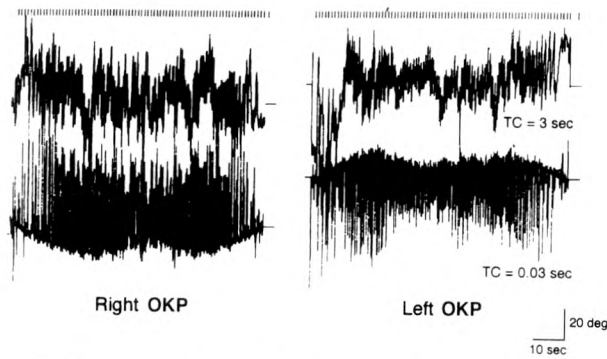


Fig 5. Horizontal optokinetic nystagmus shows left-right asymmetry. OKP—optokinetic pattern, TC—time constant.

actually presented with tinnitus of the right ear. The tinnitus improved rapidly and disappeared after 2 weeks. The ABR audiometry showed that the amplitude of waves III and IV was smaller on the left side than on the right, wave IV was nearly absent on the left, and there was prolongation of the interwave interval (Fig 3).

Impulses from the dorsal and ventral cochlear nuclei travel via a variety of pathways (both crossed and uncrossed) to the superior olivary complex, lateral lemniscus, and inferior colliculus, while other impulses enter the reticular formation.¹¹ It is assumed that the lesion in the right mid pons caused the left-sided changes of waves III and IV and the bilateral prolongation of interwave intervals on ABR testing.^{12,13} A high short increment sensitivity index score

is suggestive of cochlear disease, but is sometimes associated with cerebellopontine lesions.¹⁴ Optokinetic nystagmus showed slight left-right asymmetry (Fig 5). Lesions of the brain stem in the posterior fossa may result in a unilateral reduction of optokinetic nystagmus.¹⁵ The brain stem reticular formation has a very important role in optokinetic nystagmus,¹⁶ and is also important in the central auditory pathway.¹⁴ It is presumed that the capillary telangiectasia in the right mid pons of our patient may have affected the auditory and vestibular central pathways, causing bilateral slight sensorineural hearing loss, right-sided tinnitus, the ABR changes (abnormal left-sided waves III and IV and bilateral prolongation of the interwave intervals), and slight asymmetry of optokinetic nystagmus. Minor bleeding,¹⁷ ischemia confined to the pons (presumably secondary to a “steal” phenomenon caused by the malformation),⁸ or transient local oxygen deficiency¹⁸ may have induced the posterior fossa syndrome, including auditory and latent vestibular dysfunction.

Sudden deafness is defined as the sudden onset of hearing loss with an unknown cause. However, the incidental association of sudden deafness with capillary telangiectasia in our patient was excluded by ABR audiometry. There have been reports of hemorrhage associated with capillary telangiectasia,^{6,7} so minor bleeding may possibly be caused by these lesions.¹⁷ Vasodilators and drugs to improve blood flow, such as nicotinic acid, pentoxifylline, dextran, and

REVIEW OF CAPILLARY TELANGIECTASIA WITH HEARING LOSS, TINNITUS, DIZZINESS, VERTIGO, OR DYSEQUILIBRIUM

Case	Sex	Age (y)	Clinical Presentation	Location	References
1	F	47	Right hearing loss, vertigo, headache, intermittent gait abnormality	Right mid pons	1
2	M	53	Vertigo, tinnitus	Left rostral pons	1
3	M	69	Abrupt onset right hearing loss	Left mid pons	1
4	F	30	Recurrent vertigo, unsteadiness	Right mid pons	1
5	F	63	Transient dizziness, diplopia	Rostral mid pons	1
6	F	34	Nystagmus, dysequilibrium	Right medulla	2
7	F	77	Left tinnitus	Ventral mid pons	2
8	M	31	Dizziness, vertigo, nausea, vomiting, right-sided facial weakness	Lower mid pons, left lower pons	2
9	F	55	Hearing loss greater in right ear than in left ear, tinnitus	Left mid pons	2
10	M	55	Dizziness, dysequilibrium	Left mid pons	2
11	F	24	Imbalance, vertigo, left occipital headache, left-sided incoordination	Left cerebellar hemisphere	6
12	F	1	Mild hearing loss, febrile seizure, strabismus, ataxic gait	Midbrain, pons, medulla	8
13	M	71	Transient vertigo, diplopia	Mid pons	9
14	F	58	Dizziness, right hearing loss	Lower pons	10
15	M	36	Abrupt onset hearing loss, tinnitus	Right mid pons	Present case

urokinase, are often used for the treatment of sudden deafness.¹⁹ Therefore, in patients with hearing loss, tinnitus, vertigo, or dizziness, it is important to rule out capillary telangiectasia by MRI. If such a lesion

is found, vasodilators and drugs to improve blood flow should be avoided. In conclusion, the role of capillary telangiectasia in hearing loss, tinnitus, vertigo, or dizziness needs to be studied further.

ACKNOWLEDGMENTS — The authors thank H. Saratani and Dr H. Nakamura for their advice and help. We express gratitude to laboratory technologist K. Nakasuji and to radiological technologists H. Sasaki, H. Fukuzaki, T. Yamasaki, and T. Fukumura for their assistance.

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LETTERS TO THE EDITOR

To the Editor:

We read with interest the recent article by Drs Witak and Pensak (Limitations to mobilizing the intrapetrous carotid artery. *Ann Otol Rhinol Laryngol* 2002;111:343-8). In addition to a cadaver anatomical study, the authors report a series of 60 patients in whom they have mobilized the intrapetrous carotid artery. While we compliment the authors for their delineation of the anatomical limits of carotid mobilization, we differ on their indications for the clinical use of this technique. All of their 60 patients had benign tumors (meningioma 31, glomus 18, neuroma 6, chondroma 3, and fibroma 2). In our opinion, mobilization of the petrous carotid artery, which carries a significant risk of major stroke, is rarely if ever indicated for benign skull base tumors. In our experience, benign tumors can usually be dissected off the carotid adventitia while keeping the carotid artery in its usual anatomical position. In cases of exceptional adherence, leaving a small, well-coagulated remnant often provides long-term control. It could be argued that mobilization of the carotid artery enhances the opportunity for radical resection. However, even after carotid translocation, the authors achieved a microsurgically complete resection in only 50% (30/60). Although it was not addressed in the case series, carotid mobilization or resection in high-grade malignancies has not been proven to improve survival. In our view, the only category of neoplasms in which petrous carotid mobilization or resection may be indicated are relatively low-grade malignancies (eg, chordoma, chondrosarcoma, and certain adenocarcinomas) that have poor radiosensitivity. In general, we have reserved use of this maneuver for cases of recurrence following primary surgical extirpation. We compliment the authors on their exceptional surgical results. The absence of strokes (either thrombotic or embolic) and the lack of pseudoaneurysm formation are a testament to their meticulous surgical technique.

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To the Editor:

As noted by Dr Myers in the Editorial Comment that follows it, the recent article by Laccourreye et al (Analysis of local recurrence in patients with selected T1-3N0M0 squamous cell carcinoma of the true vocal cord managed with platinum-based chemotherapy—alone regimen for cure. *Ann Otol Rhinol Laryngol* 2002;111:315-22) has the potential to initiate “a very major paradigm shift in the treatment of early-stage cancers of the glottis without metastasis to the neck.” Recognizing that potential, I believe it is critical that all aspects of this treatment regimen be analyzed. Without doubt, the published results are excellent.

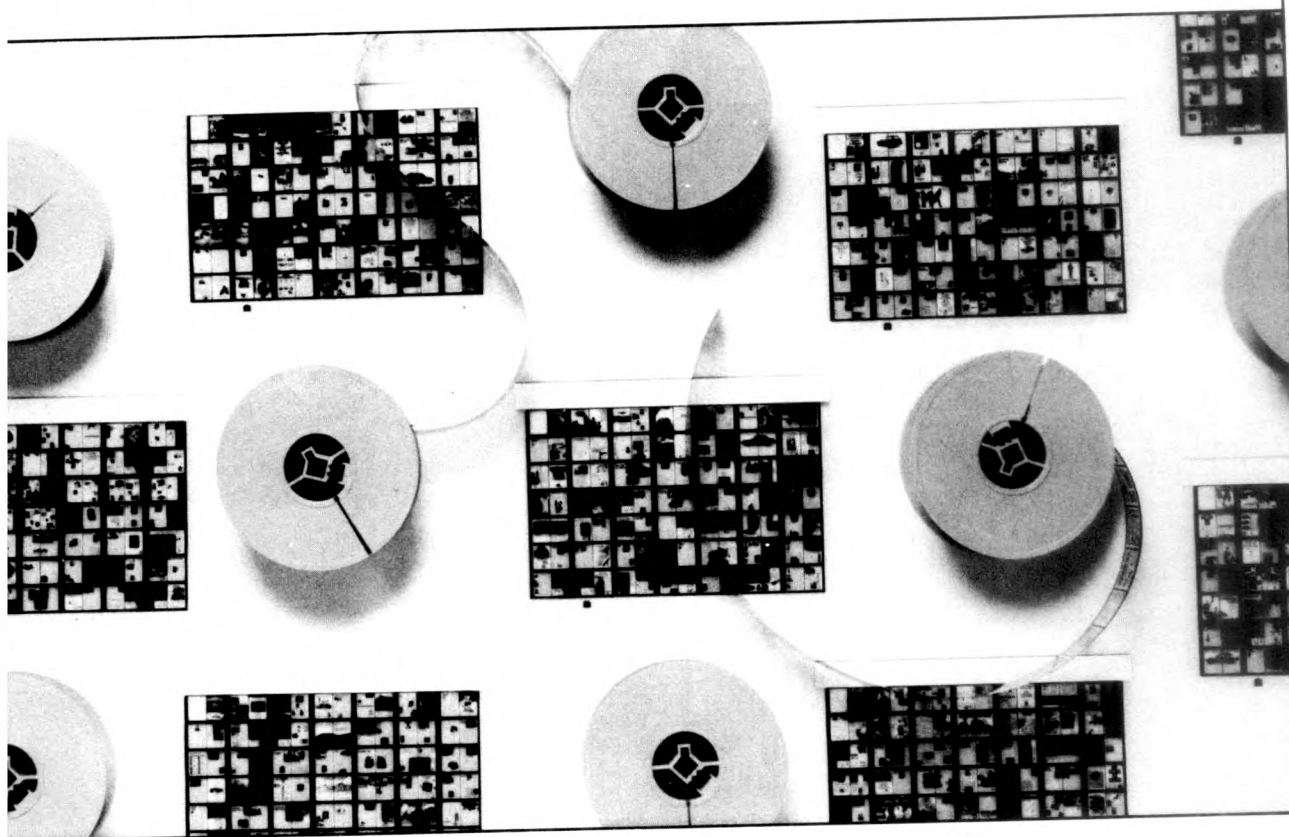
Questions that arise include the following.

1. From a group of 77 patients who achieved a complete clinical response, 35 were chosen as the cohort for this report. How were these 35 selected? What was the outcome of the remaining 42 patients?
2. Six of the 35 patients (17%) died of intercurrent disease. Is this the expected level of non-cancer mortality in this group? Or is this a manifestation of chemotoxicity?
3. Accumulating experience with long-term outcomes from chemotherapy includes a surprising proportion of patients with disabling dysphagia. What was the functional outcome with respect to swallowing in this group of patients?

Having raised these questions, I want to add my congratulations to the authors for this stimulating and potentially pivotal contribution. The next phase in its analysis should include a prospective trial.

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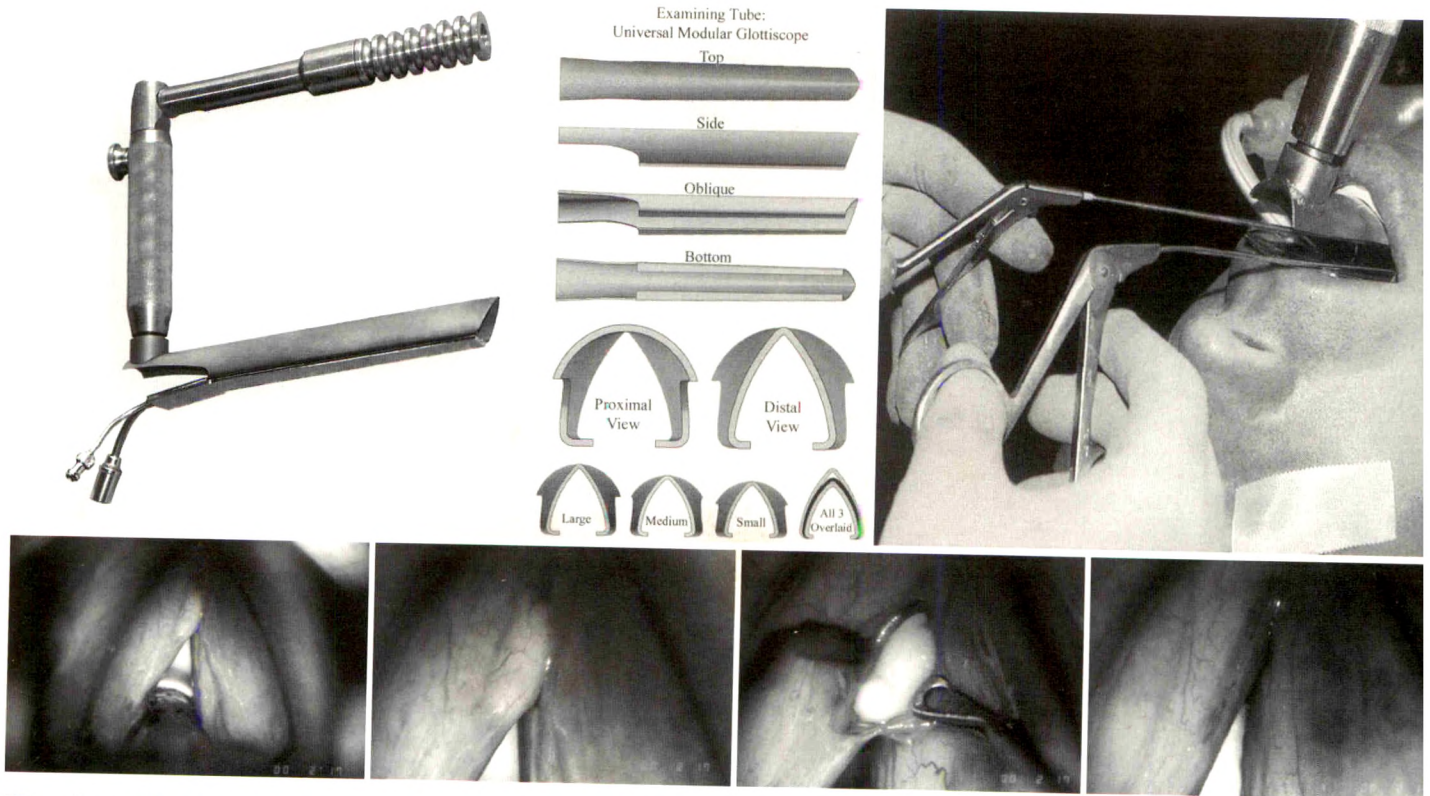


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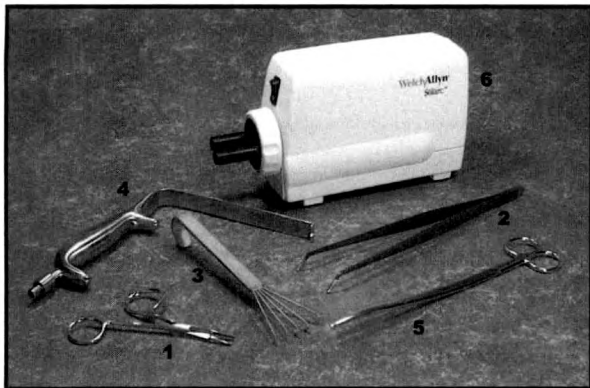
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GLOTTAL CONFIGURATION, ACOUSTIC, AND AERODYNAMIC CHANGES INDUCED BY VARIATION IN SUTURE DIRECTION IN ARYTENOID ADDUCTION PROCEDURES

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Arytenoid adduction is a phonosurgical procedure in which the arytenoid cartilages are approximated to reduce posterior glottal gap size and improve voice. Voice outcomes following arytenoid adduction are not always optimal. The goal of this study was to systematically vary suture direction and force of pull on the arytenoid cartilages in a human excised laryngeal model to determine the optimal combination of factors for reducing glottal gap and improving voice. Several factors demonstrated significant effects. Changes in suture direction and force of pull affected glottal configuration in both the horizontal and vertical planes. Increased force of pull on the muscular process resulted in increased adduction of the vocal process for all suture directions. Changes in suture direction and force of pull also affected acoustic and aerodynamic measures of induced voice. Therefore, voice outcomes can be optimized with arytenoid adduction if the vocal fold plane is accurately adjusted.

KEY WORDS — arytenoid adduction, lateral cricoarytenoid muscle, thyroarytenoid muscle.

INTRODUCTION

Among the myriad of phonosurgical procedures developed for treating unilateral vocal fold paralysis, arytenoid adduction stands out as a potentially good but underused technique. It appears to be used less frequently¹ because of its technical difficulty.^{2,3}

Historically, the surgical technique for shifting the arytenoid cartilage was first described by Morrison in 1948,⁴ and was modified in 1966 by Montgomery⁵ with a technique for fixing the arytenoid and cricoid cartilages in a new position. In 1978, Isshiki et al⁶ described arytenoid adduction as a simple and reliable surgical technique for medializing the arytenoid cartilage and improving the voice. Isshiki's original technique required attachment of two 3-0 nylon sutures to the muscular process of the arytenoid cartilage. The sutures were then inserted through different sites in the thyroid cartilage to simulate the forces of the lateral cricoarytenoid and thyroarytenoid muscles. Suture tension produced arytenoid cartilage rotation, thus medializing the vocal fold.

Arytenoid adduction, in theory, reduces glottal gap size, corrects vertical plane differences between the two vocal folds, and improves voice for patients with

glottal insufficiency. However, surgical success is not inevitable.⁷ Failure to improve voice may be due to overrotation of the arytenoid cartilage,^{2,8} shortening of the vocal fold,² and/or inadequate correction of the vertical plane differences.

Suture direction and force of suture pull may be important determinants of postsurgical glottal configuration and resultant voice improvement. Unfortunately, optimal levels for these parameters have not been identified, as there are few studies regarding laryngeal biomechanics during arytenoid adduction procedures.⁹⁻¹² Further, there are no reports in the literature of the 3-dimensional configuration changes that occur in the glottis after adjustments in arytenoid position, and no studies that systematically examined phonatory outcomes in a single larynx with systematic variations in arytenoid adduction parameters. Therefore, surgeons must make empiric adjustments in the operating room in regard to suture direction and force of pull. This method can be time-consuming and may not yield optimum results.

This study investigated the relationships among suture direction, force of pull, and 3-dimensional changes in anatomic configuration at the glottis and

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CORRESPONDENCE — Nadine P. Connor, PhD, University of Wisconsin Clinical Science Center, Room K4/711, 600 Highland Ave, Madison, WI 53792-7375.

TABLE 1. SUBJECTS

Larynx No.	Age (y)	Sex	Cause of Death	Intubation
1	79	M	Motor vehicle accident	No
2	67	M	Atrophy of cerebrum	Unknown
3	55	M	Ventricular rupture	Yes
4	66	M	Atherosclerosis	No
5	68	M	Bronchogenic cancer	No
6	43	M	Motor vehicle accident	Yes
7	31	M	Aneurysm of aorta	Yes
8	42	F	Pulmonary hypertension	Yes
9	53	F	Myocardial infarction	No
10	82	M	Bronchopneumonia	No

voice results in a human excised laryngeal model with simulated unilateral vocal fold paralysis.

MATERIAL AND METHODS

SUBJECTS

Ten excised cadavric human larynges (8 male and 2 female) were obtained from the Department of Pathology, University of Wisconsin. Six male excised human larynges (Nos. 1 to 6 in Table 1) were used to study glottal configuration changes after arytenoid adduction. The remaining 4 larynges (Nos. 7 to 10 in Table 1) were used to study acoustic and aerodynamic changes in induced phonation as a function of arytenoid adduction. The latter 4 larynges were selected for phonatory analyses because they did not have any vocal fold mucosal lesions and minimal laryngotracheal frame damage. The cause of death for each donor and the presence of an endotracheal tube at autopsy are shown in Table 1.

METHODS

Laryngeal Preparation. Extralaryngeal tissue and

muscle were dissected away to increase the stability of the larynx. For better visibility of the arytenoid cartilages and vocal fold displacement, the epiglottis and the superior part of the aryepiglottic folds were extracted without any effect on arytenoid displacement.

Arytenoid Adduction Procedure. Arytenoid adduction was performed in a manner similar to the description of Isshiki et al.⁶ The muscular process of the right arytenoid cartilage was exposed and confirmed to be mobile without intrusion at the cricoarytenoid joint. A single 3-0 Tevdek suture was tied through the exposed muscular process. As shown in Fig 1A, the sutures were passed through the larynx and emerged at 6 separate locations on the thyroid cartilage: 1) the superior notch (SN); 2) the junction of the superior edge and the anterior fourth of the superior border (FS); 3) the junction of the superior edge and the middle of the superior border (MS); 4) the inferior notch (IN); 5) the junction of the inferior edge and the anterior fourth of the inferior border (FI); and 6) the junction of the inferior edge and the middle of the inferior border (MI).

Glottal Configuration Changes After Arytenoid Adduction Procedure. The larynges were vertically mounted on a platform that stabilized both the thyroid and cricoid cartilages. For measuring arytenoid and vocal fold displacement, 5 markers were placed on the following laryngeal landmarks (Fig 1): 1) point A or the vocal process (allowing measurement of the rotation of the arytenoid cartilage and change in vocal fold length); 2) point B or the posterior end of the vocal fold (allowing measurement of the amplitude of membranous and cartilaginous vocal fold adduction); 3) point C or the tip of the arytenoid carti-

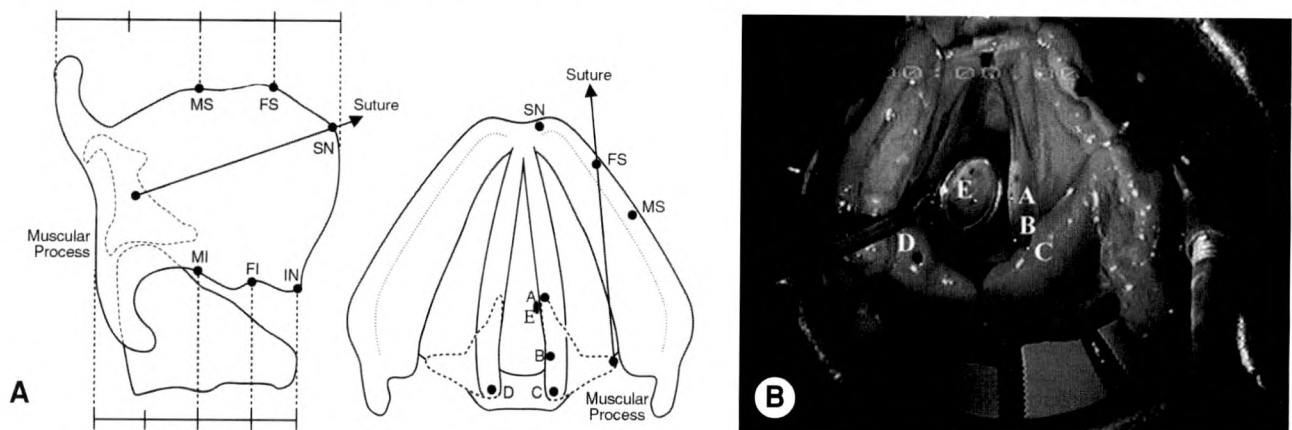
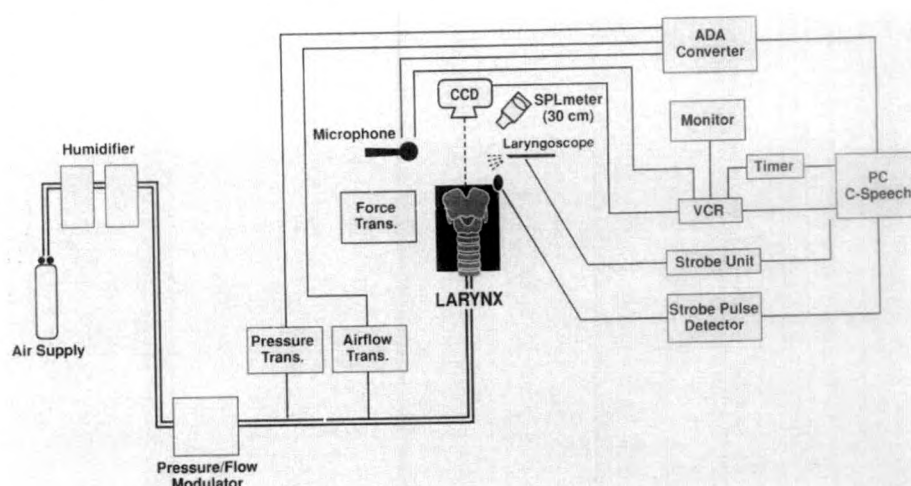


Fig 1. Schematic view (A) and superior view of excised larynx (B). Sutures attached at muscular process were directed toward 6 different directions, including superior notch (SN), anterior fourth of superior border of thyroid cartilage (FS), middle of superior border of thyroid cartilage (MS), inferior notch (IN), anterior fourth of inferior border of thyroid cartilage (FI), and middle of inferior border of thyroid cartilage (MI). Five measurement points were marked on each larynx: point A, vocal fold surface at vocal process; point B, posterior end of vocal fold; point C, tip of arytenoid cartilage at arytenoid adduction side; point D, tip of arytenoid cartilage at contralateral side; and point E, edge of vocal fold at vocal process.

Fig 2. Schematic view of excised laryngeal experimental apparatus. Compressed air was directed through humidifier and artificial lung and then released. Subglottal air pressure and airflow were transduced at position between artificial lung and larynx. Microphone was placed at 30 cm above vocal folds. Camera and stroboscopy unit positioned above larynx were used to record superior view for analysis of vocal fold vibratory characteristics. Aerodynamic and acoustic data were acquired on personal computer with available computer software.



lage (allowing examination of arytenoid tilt); 4) point D or the tip of the contralateral arytenoid cartilage (serving as a reference in video image analyses); and 5) point E or the tip of the vocal fold at the vocal process (allowing examination of the vertical displacement of the vocal process and vocal fold).

Each suture originating from the muscular process was directed to 1 of the 6 separate locations on the thyroid cartilage and was pulled with 4 different weights (20 g, 50 g, 100 g, and 150 g) for adducting the arytenoid cartilage. The weights allowed systematic manipulation of the force applied to the suture, and simulated the force of pull during arytenoid adduction procedures.

A small laryngeal mirror was placed between the vocal folds at a position in line with the anterior commissure and the midline of the posterior commissure, and was adjusted to a 45° slant relative to the vocal fold plane. The mirror allowed measurement of the vertical displacement of the arytenoid cartilage and vocal fold (Fig 1B).

A video camera was mounted above the larynx for recording a superior laryngeal view. The locations of each measurement point at both the cadavric vocal fold position and the adducted vocal fold position were recorded at each suture weight. The video images were digitized and the location of each marker (points A to E) was measured in the x and y dimen-

sions with NIH Image 1.62 software (US Department of Health and Human Services, National Institutes of Health, Bethesda, Maryland) on a personal computer.

The displacement amplitude at each measurement point was defined as the change in length (in millimeters) of the 2-dimensional displacement vector in the x- and y-axes between the cadavric and adducted positions. The displacement amplitude at point D, in x and y dimensions, was subtracted from each measure to account for translation of the whole larynx, which might be caused by pulling the suture. The arytenoid displacement amplitude (in millimeters) at measurement points A, B, and C was assessed in the lateral-medial (x-axis) and anterior-posterior (y-axis) directions. At point E, arytenoid displacement constituted a change in the superior-inferior (x-axis) and anterior-posterior (y-axis) dimensions. All measures were assessed separately at 20 g, 50 g, 100 g, and 150 g.

Phonatory Outcomes After Arytenoid Adduction. Each of the 4 larynges was mounted on scaffolding in the excised laryngeal research laboratory at the University of Wisconsin (Fig 2). The cricoid cartilage was stabilized on the horizontal plane, and the thyroid cartilage was tilted forward between 10° and 15° from the vertical plane. Compressed air was directed through a humidifier and an artificial lung and then released through the larynx. Subglottal air pressure and airflow were transduced at the position between the artificial lung and the larynx (Fig 2). A microphone was placed 30 cm above the vocal folds. A camera positioned above the larynx recorded a superior view for analysis of vocal fold vibratory characteristics. Aerodynamic and acoustic data were acquired on a dedicated personal computer with available software (C-Speech, Madison, Wisconsin).

Unilateral (left) vocal fold paralysis was simulated

TABLE 2. SHIM DEPTHS AND LOCATIONS

Larynx No.	Shim Depth (mm)	Shim Location	
		From Anterior Border of Window (mm)	From Superior Border of Window (mm)
7	6	2	0
8	3	0	1
9	5	3	1
10	5	2	1

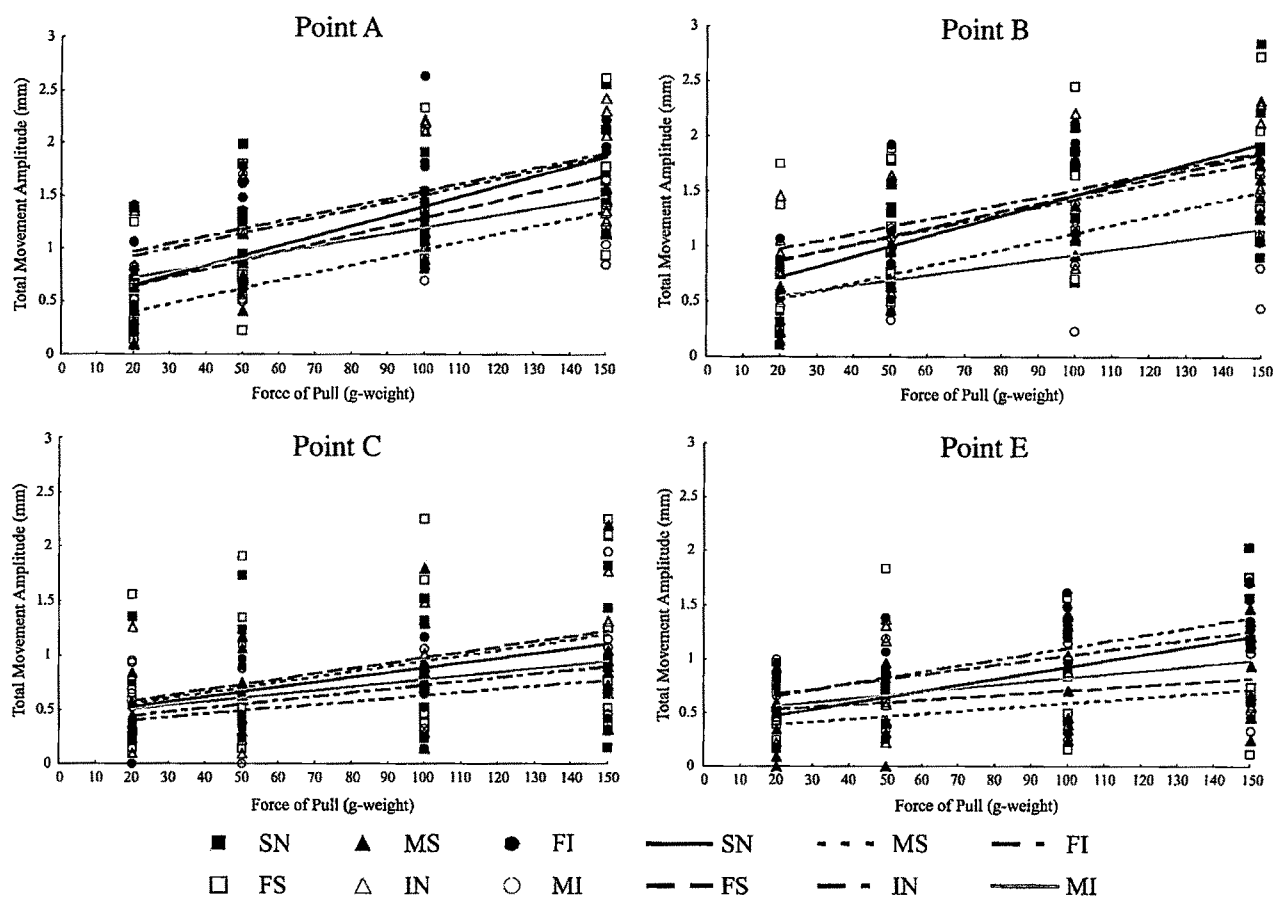


Fig 3. Relationships between total displacement amplitude and force of pull among suture directions on each point. Total vocal fold adductory amplitude and force of pull were directly proportional across suture directions and measurement points. For measurement points A and B, significant positive correlation was observed between total vocal fold adductory amplitude and force of pull in all suture directions. For measurement point C, significant positive correlation was observed between total vocal fold adductory amplitude and force of pull when suture was pulled toward only MS and FI directions. Total vocal fold adductory amplitude of point E in SN, IN, and FI directions significantly and positively correlated with force of pull.

for this arytenoid adduction study, and was treated with a left medialization thyroplasty. This was necessary to produce suitable tension of the vocal fold and to adduct the membranous vocal fold on each larynx. The thyroplasty window was 5 × 10 mm, with the anterior border located 5 mm (female larynges) or 7 mm (male larynges) from the anterior edge of the left thyroid cartilage, and the superior border located at the midportion between the superior and inferior notches. The shim sizes and locations are listed in Table 2.

The right vocal fold was adducted with a suture (3-0 Tevdek) and a fine needle. This suture was attached at the muscular process of the right arytenoid cartilage and pulled medially for simulating the function of the interarytenoid muscle. The needle was inserted lateral to the vocal fold mucosa, thus simulating the function of the thyroarytenoid and lateral cricoarytenoid muscles in inducing adduction of the vocal folds and creating vocal fold tension. It was necessary to control the position of the right vocal fold across larynges to allow comparison of phonatory out-

comes. Therefore, the right vocal fold was fixed at the same level across the 4 larynges, on the basis of phonatory outcomes with the left vocal fold adducted with sutures to both the SN and IN suture directions with 150-g weights. During monitoring of voice production on the computer monitor, the position of the right vocal fold was adjusted to a position that met acoustic criteria for the best, or model, phonation.

In this left vocal fold paralysis model, a suture from the left muscular process was directed toward 1 of the 6 thyroid cartilage positions previously described (SN, FS, MS, IN, FI, and MI), with care not to dislocate the right vocal fold position or the thyroplasty shim on the left side. The acoustic and aerodynamic properties of induced phonation were recorded at 85 dB sound pressure level (SPL). The vocal outcome was assessed with measurements of airflow, subglottal pressure, vocal efficiency index (alternating current flow divided by direct current flow), fundamental frequency (F0), jitter, percent shimmer, signal-to-noise ratio (SNR), open quotient (OQ; open phase duration divided by duration of entire cycle), speed

TABLE 3. CORRELATION (RS) BETWEEN TOTAL VOCAL FOLD ADDUCTORY AMPLITUDE AND FORCE OF PULL AMONG SUTURE DIRECTIONS

	SN	FS	MS	IN	FI	MI
Point A	0.73*	0.65*	0.88*	0.61*	0.63*	0.57*
Point B	0.67*	0.56*	0.83*	0.57*	0.63*	0.55*
Point C	0.37	0.35	0.55*	0.37	0.45†	0.37
Point E	0.62*	0.24	0.33	0.53*	0.58*	0.39

See text for definitions.

* $p < .01$.† $p < .05$.

quotient (SQ; opening phase duration divided by closing phase duration), and speed index (SI; $SI = [SQ - 1]/[SQ + 1]$).

To reduce the effect of variability of factors such as gender, age, and cause of death on measurements of vocal outcome, an improvement index was calculated for each phonatory measure within each larynx and used for comparison among suture directions. The improvement index for each of the phonatory measures was calculated as follows: Improvement index = Phonatory Measure_{SD}/Phonatory Measure_{MP}. Phonatory Measure_{SD} represents the data value obtained for each measure of phonatory function for a particular suture direction, and Phonatory Measure_{MP} is the equivalent measure recorded during production of the model phonation.

STATISTICAL ANALYSIS

Spearman correlation analysis was used to examine the relationship between the total displacement amplitude of each suture direction and the force-of-pull condition. The Spearman correlation coefficient (RS) was considered significant at a p value of less than .05.

The x-axis displacement amplitude and y-axis displacement amplitude were compared across each suture direction and weight condition. Analysis of variance (ANOVA) was used to test whether suture direction, weighting, or the interaction influenced the dependent variables. The improvement index for each phonatory characteristic was also compared across suture directions with ANOVA. If the ANOVA indicated that significant differences existed, all paired comparisons were examined with Fisher's protected least significant difference procedure (SAS, Cary, North Carolina). All testing was done at the nominal 5%; the reported p values control the comparisonwise type I error rate within each dependent variable.

RESULTS

RELATIONSHIP OF VOCAL FOLD ADDUCTION AND FORCE OF PULL

The total vocal fold adductory amplitude and the force of pull were directly proportional across suture directions and measurement points (Fig 3 and Table 3). For points A and B, a significant positive correlation was observed between total vocal fold adductory amplitude and force of pull across all suture directions. The suture directions SN, FS, IN, and FI resulted in greater displacement amplitude than suture directions MS and MI (Fig 3). For point C, a significant positive correlation was observed between total vocal fold adductory amplitude and force of pull when the suture was directed toward only the MS and FI directions. The total vocal fold adductory amplitude of point E in the SN, IN, and FI directions was significantly and positively correlated with the force of pull (Table 3).

Significant relationships were found between lat-

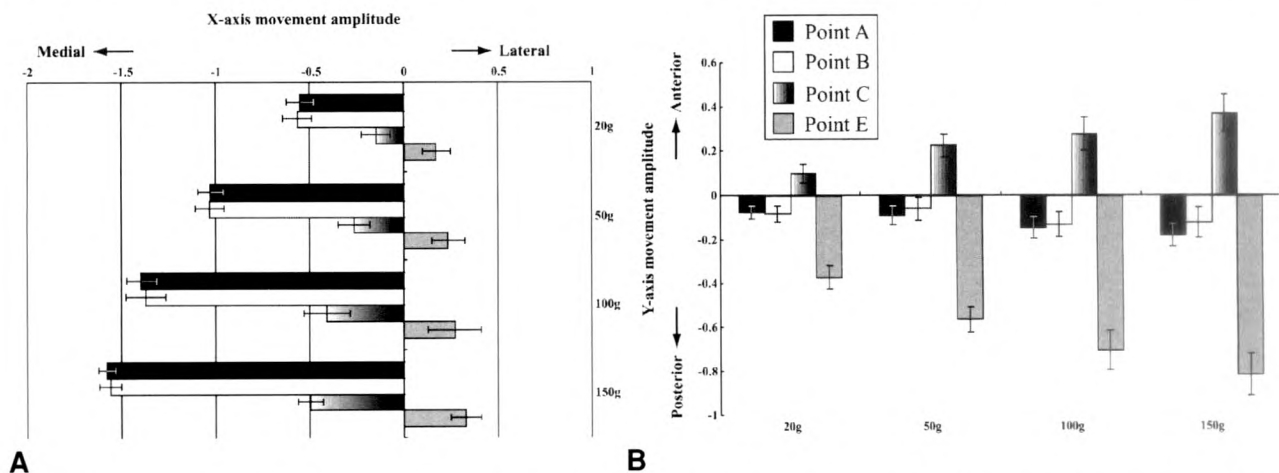


Fig 4. Comparison study showed significant relationships between lateral-medial (x-axis) displacement of points A ($p < .01$), B ($p < .01$), and C ($p < .05$) and force of pull (A). However, no significant relationship of lateral-medial arytenoid displacement was observed between 100-g and 150-g weights. For point C, medial displacement at 20 g force of pull was significantly shorter than that at 100 g ($p < .05$) and 150 g ($p < .01$). Significant relationship was observed between anterior-posterior (y-axis) displacement of point E and force of pull ($p < .01$; B). Significant differences in posterior point E displacement are noted between 20 g and 100 g ($p < .01$), 20 g and 150 g ($p < .01$), and 50 g and 150 g ($p < .05$).

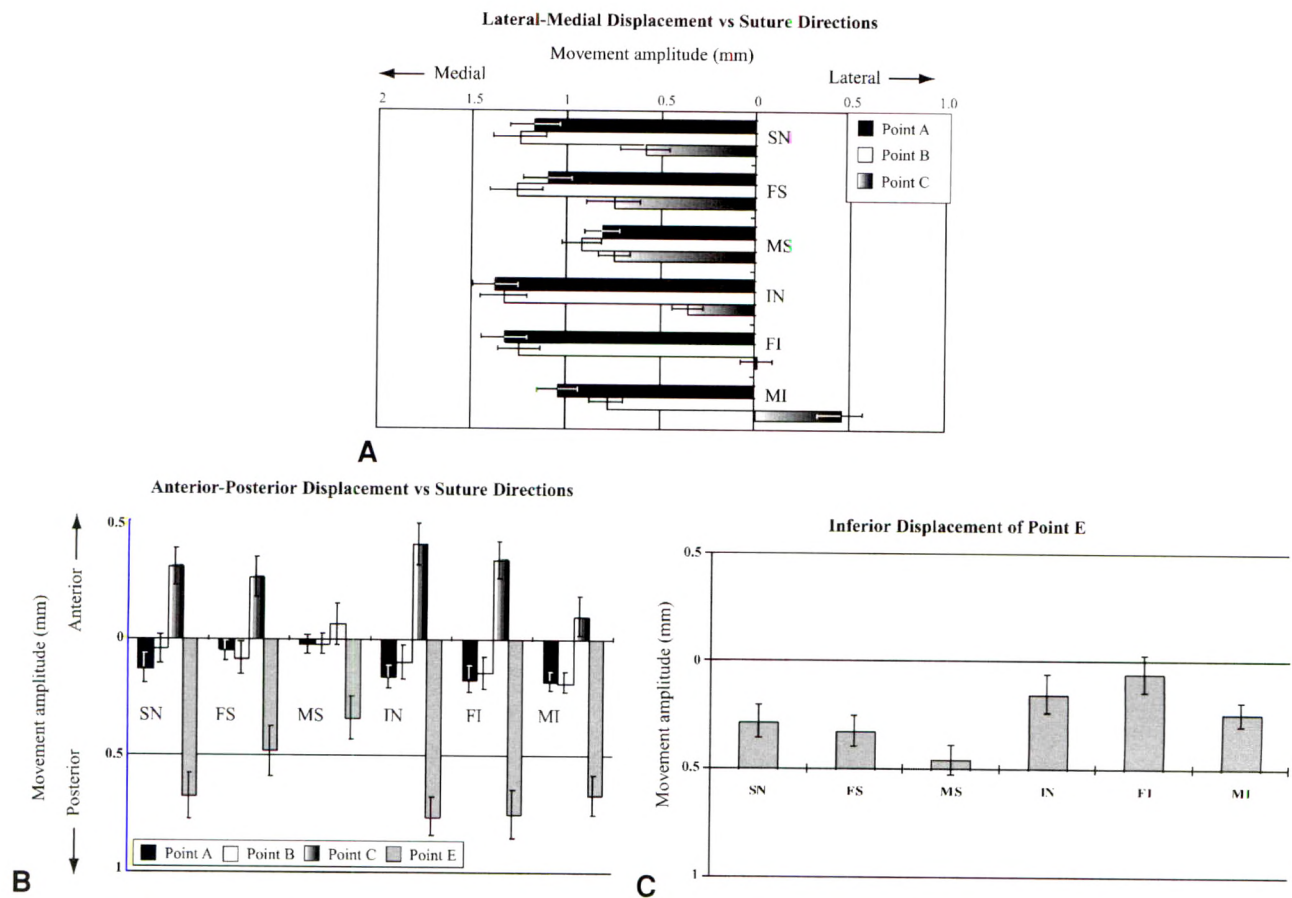


Fig 5. **A)** Lateral-medial (x-axis displacement amplitude of points A, B, and C), **B)** anterior-posterior (y-axis displacement amplitude of points A, B, C, and E), and **C)** superior-inferior (x-axis displacement amplitude of point E) arytenoid displacements. Point A was displaced medially ($p < .01$) across suture directions, with exception of suture directions FS and MS. Point A was displaced only in medial direction for FS and MS suture directions. Suture directions SN, FS, IN, and FI resulted in significantly ($p < .01$) greater adductory amplitude than MS. Point B was displaced medially (A) across all suture directions. Observing point B, suture directions SN, FS, IN, and FI resulted in significantly ($p < .01$) greater adductory amplitudes than MS and MI. For y-axis, point B was displaced posteriorly for all suture directions, except for MS (B). Average displacement amplitude of each suture direction in y-axis of points A and B was less than 0.2 mm (B). Amplitude of lateral-medial displacement (x-axis) of point C was significantly ($p < .01$) different among suture directions. Suture directions SN, FS, MS, and IN resulted in medial displacement of point C, while suture direction MI resulted in lateral displacement. In y-axis dimension, point C was displaced anteriorly across all suture directions (B). Suture directions IN and FI resulted in significantly ($p < .05$) greater anterior displacement than MS. Amplitude of anterior-posterior displacement (y-axis) of point E showed significant ($p < .05$) difference among suture directions (B). Suture directions SN, IN, FI, and MI resulted in significantly greater posterior displacement than MS. Amplitude of superior-inferior displacement (x-axis) of point E demonstrated significant ($p < .01$) difference among suture directions (C). Suture direction FI resulted in significantly greater inferior displacement than SN, FS, and MS. Suture direction IN resulted in significantly greater inferior displacement than MS. When suture direction MS was used, point E was displaced posteriorly in horizontal plane without any vertical displacement.

lateral-medial (x-axis) displacement of points A, B, and C and force of pull (Fig 4A), except at the 100- and 150-g weights. For point C, the medial displacement with 20 g of pull was significantly less than that with 100 or 150 g. A significant relationship was observed between the anterior-posterior (y-axis) displacement of point E and the force of pull (Fig 4B). Point E was displaced significantly in the posterior direction with increased force of pull.

LATERAL-MEDIAL AND ANTERIOR-POSTERIOR ARYTENOID DISPLACEMENT

Point A. Point A was displaced medially across

suture directions (Fig 5A). The suture directions SN, FS, IN, and FI resulted in significantly greater adductory amplitude than did MS. The average displacement of each suture direction in the y-axis was less than 0.2 mm (Fig 5B).

Point B. Point B was displaced medially across all suture directions (Fig 5A). The suture directions SN, FS, IN, and FI resulted in significantly greater adductory amplitudes than did MS and MI. In the y-axis, point B was displaced posteriorly for all suture directions except MS (Fig 5B). The average displacement amplitude of each suture direction in the y-axis was less than 0.2 mm (Fig 5B).

TABLE 4. ACOUSTIC AND AERODYNAMIC MEASURES FOR EACH SUTURE DIRECTION

	Model	Suture Directions					
	Phonation	MS	FS	SN	IN	FI	MI
Airflow (L/min)	30 ± 6.6	47 ± 7.4	37 ± 7.5	33 ± 7.2	43 ± 10	49 ± 9.1	56 ± 14
Subglottal pressure (cm H ₂ O)	44 ± 6.5	39 ± 2.9	40 ± 2.6	41 ± 2.7	37 ± 2.8	38 ± 3.4	37 ± 2.7
Vocal efficiency index	0.75 ± 0.03	0.63 ± 0.04	0.75 ± 0.04	0.78 ± 0.05	0.68 ± 0.05	0.61 ± 0.07	0.62 ± 0.04
Fundamental frequency (Hz)	349 ± 34.1	363 ± 25.0	358 ± 27.1	349 ± 24.0	340 ± 36.7	352 ± 32.2	351 ± 32.7
Jitter	0.014 ± 0.003	0.030 ± 0.006	0.025 ± 0.005	0.012 ± 0.003	0.021 ± 0.003	0.032 ± 0.007	0.033 ± 0.006
% Shimmer	4.0 ± 1.2	5.1 ± 1.1	5.1 ± 1.1	3.4 ± 0.82	4.4 ± 0.73	5.2 ± 1.4	5.4 ± 0.73
Signal-to-noise ratio	13.1 ± 2.20	11.4 ± 1.25	11.9 ± 1.28	14.5 ± 2.24	11.4 ± 0.88	10.4 ± 0.93	10.2 ± 1.16
Open quotient	0.06 ± 0.04	0.81 ± 0.02	0.74 ± 0.02	0.72 ± 0.03	0.74 ± 0.02	0.78 ± 0.03	0.82 ± 0.03
Speed quotient	1.29 ± 0.28	1.24 ± 0.19	1.31 ± 0.28	1.26 ± 0.34	1.10 ± 0.23	1.16 ± 0.26	1.17 ± 0.22
Speed index	-0.085 ± 0.122	-0.088 ± 0.073	-0.099 ± 0.092	-0.057 ± 0.13	-0.013 ± 0.10	-0.036 ± 0.11	-0.051 ± 0.091

Data are mean ± SD.

Point C. The amplitude of lateral-medial displacement (x-axis) of point C was significantly different among suture directions. As shown in Fig 5A, suture directions SN, FS, MS, and IN resulted in a medial displacement of point C, while suture direction MI resulted in a lateral displacement. In the y-axis dimension, point C was displaced anteriorly across all suture directions (Fig 5B). Suture directions IN and FI resulted in significantly greater anterior displacement than did MS.

Point E. The amplitude of anterior-posterior displacement (y-axis) of point E showed a significant difference among suture directions (Fig 5B). The suture directions SN, IN, FI, and MI resulted in significantly greater posterior displacement than did MS.

SUPERIOR-INFERIOR ARYTENOID DISPLACEMENT

The amplitude of superior-inferior displacement (x-axis) of point E was significantly different among suture directions (Fig 5C). Suture direction FI resulted in significantly greater inferior displacement amplitude than did SN, FS, and MS. Suture direction IN demonstrated significantly greater inferior displacement than did MS. When suture direction MS was used, point E was displaced posteriorly in the horizontal plane without any vertical displacement.

PHONATORY CHARACTERISTICS AFTER ARYTENOID ADDUCTION

Aerodynamic Measurements. The subglottal pressure needed to produce phonation at 85 dB SPL in each suture direction was between 37 cm H₂O and 41 cm H₂O (Table 4). There was not a significant difference in the improvement index of subglottal pressure among suture directions. However, the im-

provement index of both airflow and the vocal efficiency index showed significant differences among suture directions (Fig 6A). The airflow for the SN suture direction was 33 ± 7.2 L/min and was thus the lowest value among the suture directions. The highest vocal efficiency index (0.78 ± 0.05) was observed when the muscular process was directed toward the SN direction (Table 4).

Acoustic Measurements. The F0 produced by the larynges across all suture directions ranged from 340 to 363 Hz. No difference among suture directions, including the model phonation, was observed. The improvement indices of jitter and SNR were significantly different among suture directions (Fig 6B). However, no significant difference was observed in the percent shimmer improvement index. Jitter was the lowest (0.012 ± 0.003) when the muscular process was directed toward SN, and signal-to-noise ratio was the highest (14.5 ± 2.24; Table 4).

VIBRATORY CHARACTERISTICS

In Table 4, data are presented concerning measures of OQ, SQ, and SI across suture directions and the model phonation. No significant differences among suture directions were observed in these vocal fold vibratory characteristics (Fig 6C).

DISCUSSION

Thyroplasty type I and various types of vocal fold augmentation are the primary techniques used for correcting glottal incompetence. In the case of medial or lateral fixation of the vocal fold, these techniques often do not improve voice satisfactorily because of the presence of a large posterior glottal gap. The arytenoid adduction technique has been proposed as a

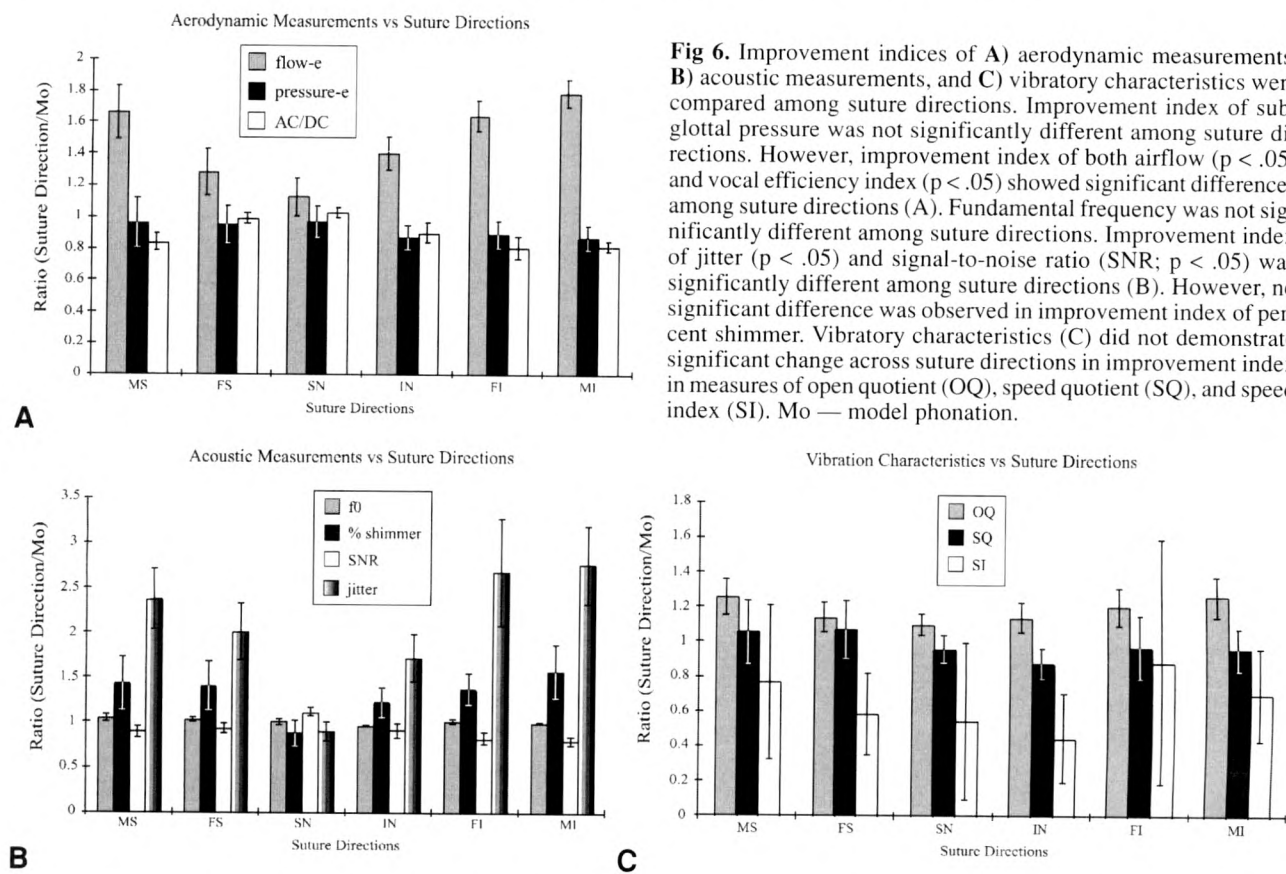


Fig 6. Improvement indices of **A)** aerodynamic measurements, **B)** acoustic measurements, and **C)** vibratory characteristics were compared among suture directions. Improvement index of subglottal pressure was not significantly different among suture directions. However, improvement index of both airflow ($p < .05$) and vocal efficiency index ($p < .05$) showed significant differences among suture directions (A). Fundamental frequency was not significantly different among suture directions. Improvement index of jitter ($p < .05$) and signal-to-noise ratio (SNR; $p < .05$) was significantly different among suture directions (B). However, no significant difference was observed in improvement index of percent shimmer. Vibratory characteristics (C) did not demonstrate significant change across suture directions in improvement index in measures of open quotient (OQ), speed quotient (SQ), and speed index (SI). Mo — model phonation.

useful procedure for patients with a large posterior glottal chink¹³ or vocal folds that are positioned off the vertical plane. For these indications, arytenoid adduction may have some advantage over other procedures, because surgical intervention does not cause trauma to the vocal fold mucosa or postoperative vocal fold scarring.

In cases of glottal incompetence, baseline arytenoid positions are highly variable in both the horizontal and vertical planes. Four of the 5 intrinsic laryngeal muscles insert into the muscular process of the arytenoid cartilage (ie, thyroarytenoid, lateral cricoarytenoid, posterior cricoarytenoid, interarytenoid). These muscles have different fiber geometries that affect arytenoid rotation. Moreover, in disordered larynges, the degree of neurologic compromise at the recurrent or superior laryngeal nerves may affect the

tension of each muscle and therefore affect arytenoid position and, ultimately, vocal fold position. Compensatory behaviors or hypertrophy often result in hyperfunction, which may create an even more difficult situation, in that vocal fold position may be more resistant to intervention. Our previous work suggests that to obtain an optimal voice outcome after phonosurgical operations such as thyroplasty type I with or without arytenoid adduction, the vocal folds must be adjusted on both the horizontal and vertical planes. The current study was designed to investigate which suture directions, when anchored at the muscular process, affect the displacement of the arytenoid cartilage and the phonatory outcome.

The underlying theory in arytenoid adduction is that the sutures attached at the muscular process physically adduct the arytenoid cartilage, instead of the

TABLE 5. INTERPRETATION OF RESULTS OF ARYTENOID ADDUCTION FOR DIFFERENT SUTURE DIRECTIONS

Suture Direction	Arytenoid and Vocal Fold Medialization	Vocal Fold Elongation	Vocal Fold Droop	Direction of Arytenoid Tilt	Vocal Outcome
FI	Good	Good	Good	Anterior	
IN	Good	Good	Good	Anteromedial	Good
SN	Good	Good	Poor	Anteromedial	Good
FS	Good	Poor	Poor	Anteromedial	
MI	Poor	Good	Good	Lateral	
MS	Poor	Poor	None	Medial	

adductory laryngeal muscles' performing this function. In their so doing, the paralyzed vocal fold is adjusted so that it is on a normal plane for phonation. However, there are conflicting findings about arytenoid movement, including that 1) the thyroarytenoid muscle is the dominant muscle for arytenoid movement¹⁴; 2) the lateral cricoarytenoid muscle is the strongest adductor for the vocal fold and the vocal process¹⁵; 3) both the thyroarytenoid and lateral cricoarytenoid muscles produce the main adductory forces and result in medial and dorsal displacement of the tip of the vocal process¹⁶; and 4) the interarytenoid muscle may be necessary to completely close the posterior glottal chink.¹⁵ The complex interaction of these muscles and the anatomic configuration of the cricoarytenoid joint, and the vectors of force applied by these muscles, are considered to be important for arytenoid movement. In the current study, we attempted to determine optimal vector direction using single sutures attached at the muscular process and then pulled to simulate specific muscles.

Increased weight on the muscular process resulted in increased adduction of the vocal process for all suture directions. As shown in Table 5, the greatest amplitudes of arytenoid displacement were obtained for suture directions FI, IN, SN, and FS, whereas vocal fold elongation was most effective with sutures directed toward FI, IN, SN, and MI. The IN, FI, and MI suture directions led to a vocal process droop with posterior displacement of the vocal fold. Pulling the sutures toward the IN, SN, and FS directions resulted in an anterior-medial arytenoid tilt, whereas pulling the suture to the MI direction resulted in a lateral arytenoid tilt with a slight anterior displacement. On the other hand, the MS suture direction tilted the arytenoid cartilage medially without any anterior-posterior displacement. Anterior displacement of the arytenoid cartilage was only observed when the FI suture direction was used. Considering these displacement effects, the surgeon might use a combination of suture directions to make fine adjustments of the arytenoid cartilage, depending upon the particular requirements of the patient's pre-surgery glottal configuration. Fine-grained arytenoid positioning may be beneficial for patients who require adjustments of both planes or of each plane independently. Sutures used in this manner provide the best control over adjustment in functional adduction of the arytenoid cartilage and in vertical positioning of both the arytenoid cartilage and the vocal fold.

Phonatory outcomes associated with different suture directions resulted in significant differences. In our left vocal fold paralysis model, the right vocal fold was adjusted to a position in which the left vo-

cal fold approximated the right vocal fold plane. Therefore, we could expect good phonatory results. Better phonatory outcomes were observed when the muscular process was directed toward the SN or IN direction as compared with other suture directions. It may be clinically significant to consider these results when performing arytenoid adduction. Our experimental model suggests that this fine-grained arytenoid positioning may be useful for patients who require better vocal quality.

In the original paper describing arytenoid adduction, Isshiki et al⁶ state that the cricoarytenoid junction should be opened, because once surgically modified, this joint may show ankylosis several months after surgery. However, opening the cricoarytenoid joint during arytenoid adduction procedures may result in a prolapse of the arytenoid cartilage,¹⁷ overadduction of the posterior commissure,¹⁷ and a shortened vocal fold because of the forward displacement of the arytenoid cartilage with traction on the suture running through the muscular process. The vocal outcome of arytenoid adduction with an opened cricoarytenoid joint may be a rough or raspy voice. In the case of ankylosis or fixation of the cricoarytenoid joint, the joint should be opened.¹³ Because an opened cricoarytenoid joint can make the movement of the arytenoid cartilage more complicated, we decided not to open the joint in this study.

There is disagreement about using an excised human laryngeal model in research, which may reduce the generalization of results to clinical conditions. The disagreement centers around the following issues: 1) clinical laryngeal paralysis does not have a complete denervation of the larynx; hence, there may be forces exerted by residual or reinnervated muscle fibers¹⁸; 2) extrinsic laryngeal muscles and contralateral intrinsic laryngeal muscles may affect the position of the arytenoid cartilage¹²; and 3) excised larynges lack support from the trachea and pharynx.¹⁹ While these issues may reflect study limitations, systematic study demands use of an excised model. It is impossible to study the arytenoid cartilage and resultant vocal fold displacements after changes in suture direction in arytenoid adduction in an *in vivo* model. Moreover, phonatory characteristics induced by changing suture direction cannot be measured in human patients under sedation.

The findings of this work provide new information about fine adjustments of both the arytenoid cartilage and vocal fold plane levels during surgery. Clinical trials based on this work are needed to fully understand the dynamic movement of both the arytenoid cartilage and the vocal fold during arytenoid adduction procedures. Surgeons must be aware of the im-

plication that suture directions have a significant impact on arytenoid adduction and resultant voice outcome.

CONCLUSION

By changing the direction of sutures attached to the muscular process, arytenoid position can be adjusted in both the horizontal and vertical planes. Fur-

thermore, by changing the direction of sutures attached to the muscular process, phonatory outcomes are dramatically improved. This fine-grained arytenoid positioning could be beneficial to patients who require adjustments of both planes or of one plane independently. Surgeons who use fine-grained arytenoid positioning should obtain more consistent vocal outcomes.

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ENDOSCOPIC BOTULINUM TOXIN INJECTION FOR CRICOPHARYNGEAL DYSPHAGIA

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Twelve patients underwent 17 endoscopic injections of botulinum toxin type A in the cricopharyngeus muscle for the treatment of dysphagia and cricopharyngeal spasm over a 3-year period. The patient's charts were reviewed. Preoperative and postoperative symptoms, examination, and swallowing studies were reviewed. Eleven of the 12 patients had improvement in their symptoms, which lasted for a mean of 3.8 months. Two patients elected cricopharyngeal myotomy for permanent correction of their dysphagia. There was 1 case of postoperative neck cellulitis in an immunocompromised patient undergoing simultaneous excision of a thyroglossal duct cyst. We conclude that endoscopic injection of botulinum toxin is a relatively safe and viable technique for the treatment of dysphagia associated with cricopharyngeal spasm. It requires simple tools readily available to otolaryngologists. Larger, prospective controlled studies are necessary to establish its effectiveness and role in the management of this condition.

KEY WORDS — botulinum toxin, cricopharyngeus, dysphagia.

INTRODUCTION

The cricopharyngeus (CP) muscle (upper esophageal sphincter) attaches to the lateral aspect of the cricoid cartilage and separates the hypopharynx from the esophagus. It is innervated by the recurrent laryngeal nerves and pharyngeal plexus. When functioning properly, this sphincteric muscle maintains esophageal closure during inspiration and between swallows, and briefly relaxes in coordination with the swallow to allow for bolus transit. It prevents the reflux of gastric and esophageal contents into the hypopharynx during inspiration. It also prevents air from entering the esophagus during inspiration. Dysphagia may result from CP muscle dysfunction.

The diagnosis of CP muscle spasm or hypertonicity begins with careful history-taking and can be confirmed with a wide variety of studies, including modified barium swallow study, esophageal manometry, and electromyography (EMG) of the CP muscle, which show persistent spasm of the CP muscle during the swallow.¹⁻⁵ Symptoms may include a cervical "block" dysphagia in which primarily solid foods get caught up at the cricoid level. Patients may also describe choking symptoms, multiple attempts at swallowing the bolus, and nasopharyngeal reflux. Consequences may include weight loss, aspiration pneumonia, episodes of asphyxia due to airway obstruction, use of alternative food consistencies, and dependence on nonoral feedings.⁶

A wide variety of disorders may affect the CP muscle and cause dysphagia. These include 1) various

neurologic disorders such as amyotrophic lateral sclerosis or multiple sclerosis, 2) cerebral vascular accidents, 3) injuries to the pharyngeal plexus of nerves or the recurrent laryngeal nerves during head and neck surgery, 4) pharyngeal diverticula, and 5) idiopathic conditions.⁶⁻⁸

Symptoms of CP dysphagia have been treated in the past with mechanical dilation, pharyngeal plexus neurectomy, and CP myotomy.^{7,9} McKenna and Dedo⁷ reported on their experience in performing 54 CP myotomies; they advocate patient selection based on careful history-taking alone. Patients with CP dysphagia may have previously had normal findings on manometric or cineradiographic studies. They advocate CP myotomy as both a diagnostic and a therapeutic modality in patients with CP dysphagia.⁷

More recently, botulinum toxin type A (Botox) has been advocated for the diagnosis and management of CP dysphagia.^{8,10,11} Botox has been used effectively for the treatment of a number of hyperkinetic disorders, such as blepharospasm, torticollis, orofacial dystonia, spasmodic dysphonia, and CP spasm that resulted in poor voice in tracheoesophageal puncture speakers.^{12,13} Botulinum exotoxin is a large protein molecule that after intramuscular injection binds rapidly and irreversibly to the presynaptic cholinergic nerve terminals. It thereby impairs the release of acetylcholine at the neuromuscular junction, causing a dose-related weakness of the innervated muscle. Its effects are mainly localized to the area in which it is administered, resulting in a process referred to as

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SUMMARY OF PATIENT DATA

Pt No.	Age (y)	Sex	Basic Disease	Modified Barium Swallow Study	Botox Dose (mouse units)	% Improvement	Duration (mo)
1	52	M	Chemotherapy and irradiation for squamous cell carcinoma of hypopharynx	Poor contraction of pharyngeal constrictors; poor relaxation of cricopharyngeus	10, 15, 20, 25	25, 25, 50, 75	3-6
2	72	M	Cerebrovascular accident	Pooling in pyriform sinuses	15	50	3
3	58	M	Idiopathic	Poor relaxation of cricopharyngeus	15	50	4
4	76	M	Idiopathic	Hypopharyngeal pooling	15	25	3
5	63	M	Total laryngectomy for squamous cell carcinoma of larynx	Poor relaxation of cricopharyngeus	15	75	6
6	69	M	Total laryngectomy for squamous cell carcinoma of larynx	Poor relaxation of cricopharyngeus	15, 30	50, 75	4
7	71	F	Amyotrophic lateral sclerosis, dysphagia	Poor relaxation of cricopharyngeus	25	25	4
8	75	M	Cerebrovascular accident, unilateral true vocal fold motion impairment	Poor relaxation of cricopharyngeus	15, 30	50, 75	4
9	58	M	Total laryngectomy for squamous cell carcinoma of larynx	Poor relaxation of cricopharyngeus	20	75	6
10	86	F	Parkinson's disease, small vessel disease, arteriosclerosis	Pharyngeal dysphagia	15	25	3
11	80	F	Idiopathic cervical dysphagia	Poor relaxation of cricopharyngeus	15	100	4
12	69	F	Idiopathic cervical dysphagia	Poor relaxation of cricopharyngeus	20	No follow-up	No follow-up

chemical denervation. Additionally, its therapeutic effects are proportional to the dose, allowing for treatment to be tailored to the patient and disorder. Therapeutic effects are usually seen within 3 days of injection. Peripheral neuronal sprouting prevents these therapeutic effects from being permanent; reports suggest clinical efficacy lasting up to 6 months.¹⁴⁻¹⁶

In the current literature, the treatment of CP dysphagia with Botox has been described in a small number of patients with short follow-up; both percutaneous and endoscopic techniques were used.^{8,10,11} In the current study, we set out to review our experience over the past 3 years with endoscopic injection of botulinum toxin for the treatment of dysphagia related to CP muscle dysfunction.

MATERIALS AND METHODS

Design. A search of the otolaryngology patient database identified 12 patients who underwent endoscopic injection of botulinum toxin into the CP muscle between July 1997 and June 2000. All patients were referred by a speech and language pathologist after modified barium swallow study. A retrospective chart review was performed of these patients. Preoperative and postoperative symptoms, examination findings, and videofluoroscopic swallowing study findings were reviewed.

Technique. All procedures were performed in the

operating room under a brief-acting general anesthetic. An adult Dedo laryngoscope was inserted into the upper esophagus, and the patient was suspended with a Lewy suspension apparatus on a Mayo stand with the CP muscle in view. A 7-inch, 22-gauge spinal needle (Becton Dickinson and Co, Franklin Lakes, New Jersey) attached to a 3-mL Luer Lock syringe was inserted under direct visualization into the CP muscle. Freshly reconstituted, purified botulinum toxin type A (Botox, Allergan Inc, Irvine, California) at a 2.5-mouse units (MU)/0.1 mL concentration was injected into 3 sites — 1 posterior and 2 lateral — in the muscle after initial aspiration to ensure that the needle was not placed in an intravascular site. The patients were observed after operation in the recovery room and subsequently discharged home. A postoperative modified barium swallow study was performed between 2 and 3 weeks after the procedure. The patients were asked to return for follow-up at 1 month and again when their swallowing function had returned to the preoperative level. At follow-up, they were asked to rate the level of improvement in their swallowing function as compared to their preoperative state by quartiles, taking into account the consistency of food consumed, the length of time required for meals, and coughing and choking episodes during meals.

RESULTS

The Table summarizes the clinical results of the

patients reviewed. There were 8 men and 4 women. Their ages ranged from 52 to 86 years, with an average age of 68 years. There were 3 patients who had undergone total laryngectomy for squamous cell carcinoma of the larynx, and 1 patient who was treated with chemotherapy and irradiation for hypopharyngeal carcinoma. There were a total of 4 patients with neurologic causes for CP dysphagia: 2 with cerebrovascular accidents, 1 with amyotrophic lateral sclerosis, and 1 with Parkinson's syndrome and small vessel atherosclerotic disease. There were 4 patients with idiopathic cervical dysphagia; 1 had undergone right upper lobectomy for carcinoma, but had normal true vocal fold function. The most common finding on preoperative swallowing study was a narrow upper esophageal sphincter with anterior bulging of the CP muscle and failure to relax during swallowing. Other findings included pooling of secretions in the hypopharynx and poor contraction of the pharyngeal constrictor muscles.

Three patients underwent multiple Botox injections, whereas the remaining 9 patients underwent 1 procedure each. The doses of botulinum toxin ranged from 10 to 30 MU, with an average dose of 19 MU. All patients were seen in follow-up at 1 month after operation and then again when symptoms of preoperative dysphagia returned. One patient was lost to follow-up. All patients reported between 25% and 100% improvement in their swallowing function as compared to their preoperative status, with an average improvement of 53%. This improvement included less difficulty passing the bolus, faster consumption of food, and less coughing and choking during meals. The duration of improvement was between 3 and 6 months, with an average duration of 3.8 months. Postoperative modified barium swallow study was performed after 11 of the 17 injections. In 8 of these, there was no obvious difference from the preoperative examination.

One patient, who underwent concomitant excision of a thyroglossal duct cyst, developed neck cellulitis after operation that resolved with intravenous antibiotic therapy. One patient did not come for any postoperative follow-up. Two patients elected to undergo CP myotomy for permanent correction of their dysphagia. Four patients received additional injections periodically—1 of these after the study period ended. Of the remaining 5 patients, the 3 with only 25% improvement in their symptoms chose not to have further therapy, and 2 died of unrelated causes.

DISCUSSION

Botulinum toxin injection into the CP muscle has been a useful addition to the otolaryngologist's arma-

mentarium for the treatment of cervical dysphagia. Schneider et al¹⁰ first injected Botox for the treatment of CP dysphagia in 1994, using EMG guidance with endoscopy in 7 patients. Five of their 7 patients subjectively experienced complete relief or marked improvement in their swallowing symptoms. Blitzer and Brin¹¹ in 1997 described a percutaneous, EMG-guided approach for Botox injection as being both diagnostic and therapeutic for this condition. All 6 patients in the study had improvement as judged by decreased pooling on the modified barium swallow study.

Both of these groups relied on EMG, which may not be available to most practitioners. In addition, the percutaneous approach may not be reliable in inexperienced hands and is somewhat uncomfortable for patients because it requires rotation of the larynx; it is also very difficult to locally anesthetize the entire path of injection. Ahsan et al⁸ more recently described an endoscopic approach used without EMG in a limited number of patients.

Since 1996, the senior author (A.M.S.S.) has been endoscopically injecting Botox into the CP muscle without EMG by the technique described. We reviewed our series of 12 patients who underwent 17 injections between 1997 and 2000. Eight of them reported 50% or greater subjective improvement in their swallow function on a quartile scale. Three patients reported only 25% improvement; 2 of them were elderly patients with chronic neurologic disease, and the third had idiopathic CP dysfunction. There was only 1 complication: neck cellulitis associated with a concomitant Sistrunk procedure in an immunocompromised patient.

Postoperative modified barium swallow examination failed to show any significant difference from the preoperative state in 8 of 11 studies. The patients, however, very consistently reported improvement in their swallowing function as starting within 1 week after injection and disappearing 4 to 6 months after injection. This apparent discrepancy may indicate that the modified barium swallow study may not be sensitive enough to determine significant differences in the swallowing function that patients were clearly able to notice. A more quantitative evaluation of swallowing, such as scintigraphy, might have been able to determine postoperative changes. Such an evaluation would allow more objective diagnosis of the swallowing dysfunction before operation, as well. Investigation into this use is under way at our institution.

Evaluation of swallowing symptoms in our patients was performed with a series of questions regarding the tendency of the bolus to become "stuck"

or impeded, and the amount of choking and coughing that takes place with food ingestion. A more extensive, statistically validated questionnaire would be more helpful in evaluating dysphagia patients.

More recently, we have been performing these procedures without an endotracheal tube, using mask ventilation and apneic technique. This practice has further reduced operative time, to less than 10 minutes on average.

CONCLUSIONS

In our series, the safety and viability of endoscopic

injection of botulinum toxin in the treatment of dysphagia related to CP muscle dysfunction was examined. Our results suggest that endoscopic Botox injection for the treatment of CP muscle dysphagia was both relatively safe and efficacious in a significant majority of our patients. This relatively simple procedure may be performed with instruments readily available to and techniques well known to most otolaryngologists. Larger, prospective, controlled trials are necessary to establish the effectiveness and role of the endoscopic technique in the management of this condition.

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EFFECT OF SUBSTANCE P INJECTION INTO THE NUCLEUS TRACTUS SOLITARIUS OF RATS ON CRICOTHYROID AND THYROARYTENOID MOTOR ACTIVITY AND CARDIOVASCULAR AND RESPIRATORY SYSTEMS

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Identification of central neurotransmitters that mediate laryngeal adductor and/or tensor activity may prove useful in managing pathological laryngeal adduction as occurs in laryngospasm or apparent life-threatening events. The putative transmitter substance P (SP) is found in the nucleus tractus solitarius (NTS), in which laryngeal afferents terminate. Therefore, we studied the laryngeal, cardiovascular, and respiratory effects of SP injected into the NTS of rats. We completed bilateral stereotactic injections of 20 nL of SP (15 μ mol) or control solution into the region of the NTS, the dorsal motor nucleus (DMN), or the nucleus gracilis (GR) in 30 anesthetized rats. Changes in diaphragm, cricothyroid (CT), and thyroarytenoid (TA) electromyography (EMG), as well as blood pressure (BP), were compared. The injection sites were verified histologically. Injection of SP into the NTS altered CT and/or TA EMG activity in all animals. The change ranged from complete inhibition, to a phasic increase, to a tonic increase. No change in laryngeal adductor EMG activity was seen in 8 of 9 animals after SP injections into the DMN (4/5) or GR (4/4), but 1 animal demonstrated brief inhibition of CT and TA EMG activity after SP injection into the DMN. Injection of SP into the NTS induced central apnea and a significant decrease in BP in all animals. The duration of apnea tended to be longer after NTS injections than after DMN or GR injections ($p < .10$ and $p < .05$, respectively). We conclude that stereotactic injections of putative neurotransmitters in rats may be accomplished to identify effects on laryngeal motor activity. Direct application of SP into the NTS consistently elicits a change in CT and/or TA EMG activity, ranging from inhibition to excitation. This model may prove useful in evaluating pharmacological targets of central reflex activity to manage life-threatening laryngeal reflex activity.

KEY WORDS — cricothyroid muscle, laryngeal adduction, laryngospasm, nucleus tractus solitarius, substance P, thyroarytenoid muscle.

INTRODUCTION

Stimulation of the laryngeal or esophageal mucosa causes reflex vocal cord adduction that is thought to protect against aspiration of material into the tracheo-bronchial tree. At times, this protective reflex becomes pathological and manifests as laryngospasm or as other reflexes such as the esophagolaryngeal adductor reflex (ELAR).¹

The ELAR is induced by stimulating sensory nerve endings in the distal esophagus and manifests as inspiratory stridor from vocal cord adduction or as obstructive apnea. The existence of the ELAR may explain how distal esophageal reflux can elicit changes in laryngeal motor activity in the absence of direct laryngeal stimulation. In dogs, the ELAR is far more

pronounced in young animals (7 to 19 weeks of age) than in mature animals.¹ This age predilection parallels pathological conditions such as reflux-related apnea and perioperative laryngospasm, both of which occur far more frequently in infants and young children than in adults.

Surgeons and anesthesiologists recognize that reflex laryngeal motor activity can be prevented by administration of neuromuscular blocking agents such as succinylcholine, which functions at the motor end plate. The mediators, however, that elicit or moderate reflex laryngeal motor activity *centrally* are poorly understood. We have previously demonstrated that intravenous substance P (SP), a sensory neurotransmitter, evokes laryngeal motor activity and that the

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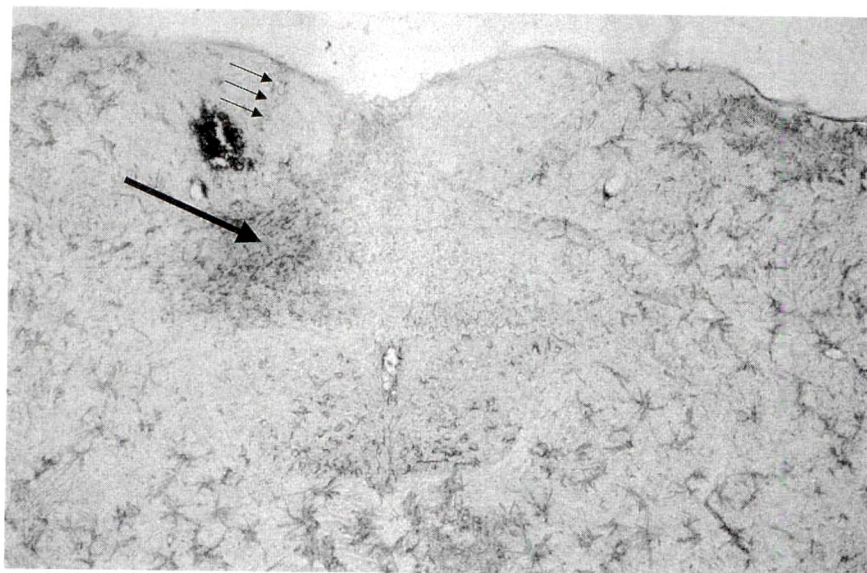


Fig 1. Axial section of rat brain stem at caudal level of area postrema after injection of 10 nL of pontamine sky blue into region of nucleus tractus solitarius (NTS). Small arrows — tract of injecting micropipette; large arrow — region of pontamine sky blue injection. Note “halo effect” of indicating dye.

response is particularly pronounced in developing animals.² The presence of immunoreactivity to SP in the nucleus tractus solitarius (NTS),³ the sensory relay center of the larynx, suggests that this neuropeptide may play a critical role in mediating or modulating laryngeal reflex activity centrally.

In dogs, we have shown that SP is a mediator of the ELAR and that injection into the region of the NTS in developing animals evokes an increase in laryngeal adductor and/or tensor activity and cardiovascular changes.⁴ In this study, we characterized the laryngeal and cardiovascular responses to SP injection into the NTS of rats. This model is useful for characterizing the neurotransmitters involved in eliciting laryngeal reflex activity and may ultimately reveal targets for pharmacological treatment of life-threatening laryngeal reflex disorders.

MATERIALS AND METHODS

Studies were completed on 30 Sprague-Dawley rats, 101 to 233 days of age (mean, 149 days), with a weight of 400 to 600 mg. Twenty-seven rats were male. The rats were initially anesthetized with 1.0 mL/kg of intramuscular ketamine with xylazine (87 mg/mL and 13 mg/mL, respectively) and then maintained with intravenous α -chloralose (20 mg/kg per hour). The rats breathed spontaneously through a tracheal cannula inserted between the third and fourth tracheal rings. Cardiovascular changes were monitored via a femoral arterial catheter attached to a pressure transducer.

Bipolar, stainless steel, hooked wire electrodes (0.003-inch-diameter, 1-mm hook) were placed into the thyroarytenoid (TA) and cricothyroid (CT) muscles bilaterally and into the diaphragm (DP). Activity from the electromyography (EMG) electrodes and pressure transducer were viewed on-line via a com-

puter monitor with data acquisition software (Win-daq). Signals were low-pass-filtered at 500 Hz and then digitized at a sampling rate of 1 kHz per channel and transferred to a magnetic optical disk for subsequent analysis.

The animal was placed prone in a stereotactic frame (model 1520, Kopf Instruments, Tujunga, California) with the head inclined downward 45°. The caudal midline cerebellum was gently retracted superiorly through an occipital craniotomy to expose the dorsal medulla. The dura was incised, and the calamus scriptorius, the point on the dorsal surface of the brain stem at which the area postrema ends caudally, was used as the zero reference point.

A 3-barrel glass micropipette (outer diameter, 50 μ m) was filled with 3 solutions: 1) a test solution of SP (Sigma Chemical, St Louis, Missouri, S6883) in 0.9 normal saline solution with 1% human albumin, titrated to pH 7.4 (15 pmol/20 nL); 2) a control solution of artificial cerebrospinal fluid, pH 7.4, or SP solvent, pH 7.4; and 3) an indicating dye solution of pontamine sky blue or methylene blue diluted in 0.9% normal saline solution.

Twenty nanoliters of SP solution or control solution were delivered pneumatically into the region of the NTS (Picospritzer, PV 800, World Precision Instruments) at the following coordinates from the calamus scriptorius: anterior-posterior, 0.4 mm; medial-lateral, 0.5 mm; and dorsal-ventral, 0.5 mm.

The order of injection was alternated between the left and right brain stem regions and between control and test solutions for the animals, and the responses were recorded. Glutamate was used in the early studies to attempt to localize the injection site of interest within the NTS, but the excitatory amino acid failed to reliably induce CT or TA EMG activ-

CHANGES IN CT AND/OR TA EMG SIGNAL FOLLOWING SP INJECTIONS INTO NTS, DMN, OR GR

Injection Site	Tonic Increase	Marked Phasic Increase	Mild Phasic Increase	Silence	No EMG Change
NTS (n = 15)	7	2	5	1	
NTS + DMN (n = 9)	3	2	2	2	
NTS + GR (n = 3)	1		2		
DMN + NTS (n = 10)	2	5	1	2	
DMN (n = 5)				1	4
GR (n = 4)					4
GR + NTS (n = 5)				2	3

Based on diffusion of marking dye, injection sites sometimes overlapped adjacent nuclei. In such cases, both nuclei are listed, and first indicates location of central point of dye halo. Changes in laryngeal electromyography (EMG) activity ranged from tonic contraction, to phasic increase, to complete inhibition of signal. Injections into region of nucleus tractus solitarius (NTS) consistently elicited change in laryngeal EMG activity, while injections into nucleus gracilis (GR) and dorsal motor nucleus (DMN) did not. CT — cricothyroid muscle; TA — thyroarytenoid muscle; SP — substance P.

ity and was deleted from the protocol. The reason for this finding was unclear, but was perhaps due to the induction dose of ketamine, a potent *N*-methyl-D-aspartate receptor blocker.

Some animals required manual resuscitation to prevent death from sustained hypotension and/or central apnea induced by injection of SP.

The injection site of each rat was marked with 10 nL of pontamine sky blue or methylene blue, and the rats were euthanized with intravenous Nembutal (80 mg/kg; Fig 1). Early attempts at injecting 20 nL of indicating dye resulted in extrusion of the dye onto the surface of the brain stem — a phenomenon that did not occur after injection of the same volume of SP or the control solutions. The brain stems were immediately harvested upon euthanizing the animals, fixed in a solution of formalin (10%) and sucrose (25%) for a minimum of 48 hours, and then sectioned on a cryostat (40 to 50 μ m). The injection sites were identified on non-counterstained transverse sections of the brain. The location of the injected dye was used to identify the central point of injection.

Mean blood pressure (BP) was calculated by averaging diastolic BP of 3 consecutive waveforms. The percent change in mean BP compared to baseline measurements (mean baseline BP minus mean nadir BP divided by mean baseline BP) was analyzed for the various test solutions and injection sites with an analysis of variance (ANOVA) and Student's *t*-test.

The duration of central apnea was calculated in seconds from the onset of cessation of DP EMG activity to either resumption of DP EMG activity or, in some animals, to the start of resuscitation. The difference in duration of central apnea in seconds was also compared by an ANOVA and Student's *t*-test.

Changes in the CT and TA EMG signals were classified as “decreased” or “increased” in comparison to the phasic inspiratory activity accompanying DP EMG activity during baseline recordings. Increased EMG activity was further subdivided into a “phasic increase” if CT and TA EMG activity was present during inspiration and absent during expiration, or a “tonic increase” if EMG activity was continuous throughout multiple inspiratory and expiratory cycles. Although direct laryngoscopy was not completed in these small, prone rodents in the stereotactic frame, previous studies on dogs and pigs in our laboratory confirmed tonic CT and TA EMG activation to correlate with visible laryngospasm.^{1,2,5}

If responses of the CT and TA muscles varied within the same animal during a study, the response was categorized by the more robust activity observed. For example, if an injection induced tonic activity in the ipsilateral TA muscle but only a phasic increase in the contralateral CT and ipsilateral CT and TA muscles, the response was categorized as a tonic increase.

RESULTS

HISTOLOGIC ANALYSIS OF INJECTION SITES

Results were assessed in 51 of the potential 60 studies performed in the 30 animals. In the remaining 9 studies, either the animals died before the second side was injected or histologic analysis was inadequate to ascertain the site of injection. As is evident in Fig 1, the dye volume diffused beyond the injection site. The diffusion pattern was spherical, extending not only in medial-lateral and dorsal-ventral directions as shown in Fig 1, but also in a cranial-caudal direction. The diffusion sphere or “halo” of the 10 nL of the marking dye overlapped adjacent brain stem nuclei in some cases. Because of this spherical diffusion phenomenon, injections in which the central point was in the NTS (27) were further subdivided into injections in which the halo was contained completely within the NTS (15); predominantly within the NTS but with overflow into the dorsal motor nucleus (DMN; 9); or predominantly within the NTS but with overflow into the gracilis nucleus (GR; 3; see Table). Injections into the DMN (15) and the GR (9) were similarly noted and subdivided.

RESPONSE TO SUBSTANCE P INJECTIONS

Laryngeal Response. The responses of the adductor TA and/or CT muscles to SP injection are sum-

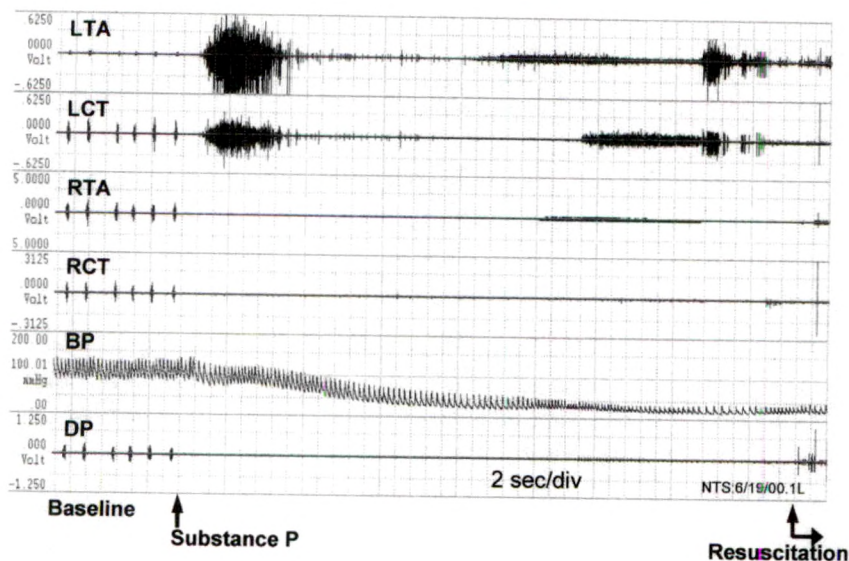


Fig 2. Response to substance P (SP) injection into left NTS. Tonic contraction of left thyroarytenoid (LTA) and left cricothyroid (LCT) muscles accompanied cessation of electromyography (EMG) activity in right thyroarytenoid (RTA) and right cricothyroid (RCT) muscles. Laryngeal response was accompanied by prolonged central apnea, as noted by absent diaphragm (DP) EMG signal, and marked decrease in blood pressure (BP) necessitating animal resuscitation.

marized in the Table. All 27 studies whose central point of dye injection was in the NTS demonstrated a change in CT and/or TA EMG activity. The EMG activity increased in 24 of the 27 studies, and in 3 studies, the EMG signal ceased. The following patterns of CT and TA EMG responses were observed: tonic increase (Fig 2; $n = 11$); marked phasic increase (Fig 3; $n = 4$); minimal phasic increase (Fig 4; $n = 9$); and EMG silence ($n = 3$).

The increase in CT and TA EMG activity was always heralded by a brief period of EMG silence (inhibition).

The laterality of the response of the CT and TA muscles to SP injection into the NTS was variable.

The ipsilateral CT and TA muscles usually demonstrated the greatest increase in EMG activity, but in some animals, the response was more pronounced in the contralateral CT and TA muscles (Fig 3). Bilateral responses tended to occur more frequently in cases of marked laryngeal responses characterized by tonic contraction. Interestingly, the laryngeal adductor muscles appeared capable of responding to SP injection independently of each other; some animals demonstrated excitation in 1 or more muscles and inhibition in the remaining muscles (Fig 2). A recognizable pattern of laryngeal motor response based on order of injection or central site of injection within the NTS was not evident, despite an exhaustive analysis of the data.

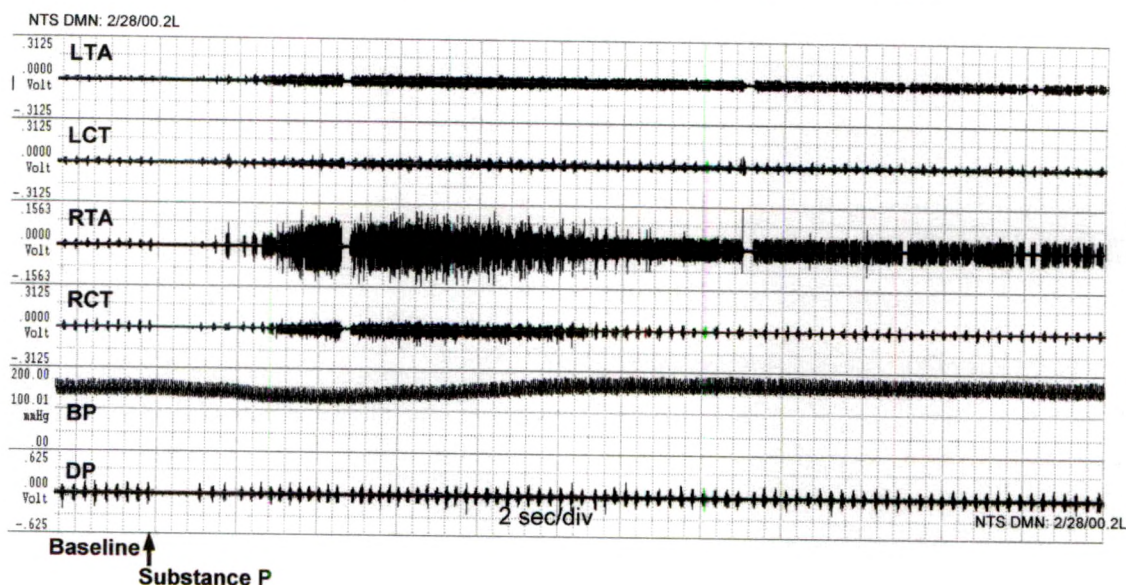


Fig 3. Response to injection of ventral aspect of left NTS and dorsal motor nucleus (DMN). Note brief absence, followed by marked tonic increase in RCT, RTA, and LTA EMG activity, and phasic increase in LCT EMG activity. BP initially decreased, then rose above baseline BP.

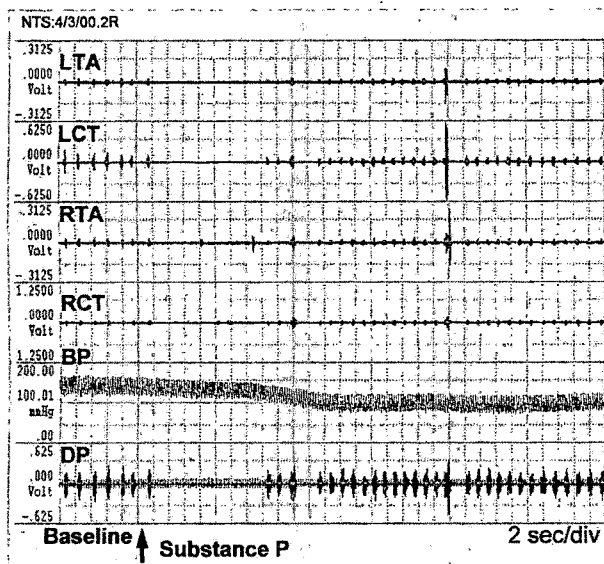


Fig 4. Response to SP injection into right NTS. Note initial inhibition of all CT and TA EMG activity, followed by increase in respiratory rate and mild phasic increase in RCT EMG signal. BP decline was moderate.

Although cardiovascular changes were usually observed, SP injections that were contained within the GR or DMN failed to elicit an increase in CT or TA EMG activity in 4 of 4 and 4 of 5 animals, respectively (Figs 5 and 6). Injection of SP into the region of the DMN of the remaining animal caused a brief period of silence in CT and TA EMG activity. A change in laryngeal adductor EMG activity occurred in all animals in which the central point of injection was in the NTS, even if the diffusion sphere of the dye extended into the DMN or GR.

Laryngeal responses for injections at the level of the area postrema (38/51) were plotted on a transverse projection of the brain stem (Fig 7). As previously noted, the dye diffused in a spherical pattern, and only the central points of the diffusion spheres are indicated in Fig 7. The CT and TA EMG responses to injections rostral to the area postrema ($n = 4$) and caudal to the area postrema ($n = 9$) are not depicted in Fig 7, but did parallel the responses observed for the same nuclei at the level of the area postrema. The responses at the level of the area postrema and depicted in Fig 7 are as follows. Injections in which the central point of the dye sphere was contained in the NTS consistently elicited a change in laryngeal adductor EMG activity. A tonic increase in activity was only observed after injections into the NTS. Injections in which the central point of the dye sphere was contained within the GR failed to elicit a change in CT or TA EMG activity, demonstrating neuroanatomic specificity of response. Similarly, injections of SP into the DMN did not elicit changes in 4 of 5 studies in which the injections were in the dorsal as-

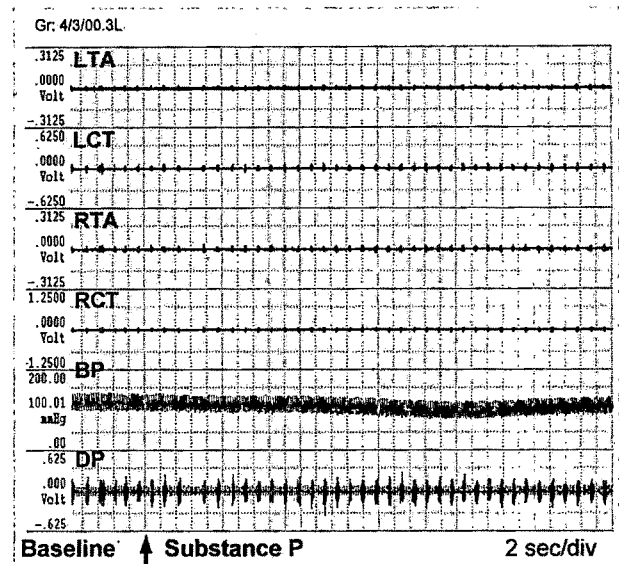


Fig 5. Response to SP injection into left nucleus gracilis (Gr). Note absence of change in CT, TA, and DP EMG activity. Minimal decline in BP was observed.

pect and the dye halo was contained within the DMN. Injections at the ventral aspect of the DMN with overflow of the dye halo into the NTS, however, did evoke changes in CT and/or TA EMG activity in 8 of 10 studies.

Apnea Response. Central apnea was inferred from the absence of DP EMG activity. The mean duration of apnea was greater in animals that received injections into the NTS than in those that received injections into the DMN ($p < .10$) or the GR ($p < .05$; Fig 8). Resuscitation was required in some of the NTS-injected animals, artificially terminating the duration

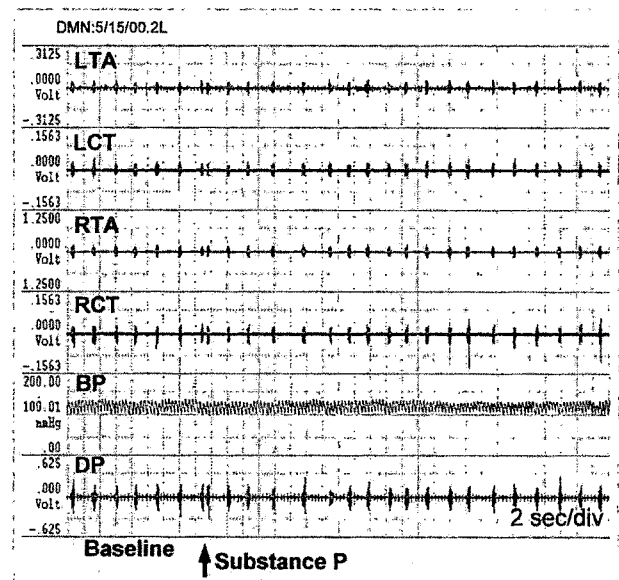


Fig 6. Response to SP injection into left DMN. Note absence of change in EMG activity of RTA, RCT, LTA, and LCT muscles. BP decrease was minimal.

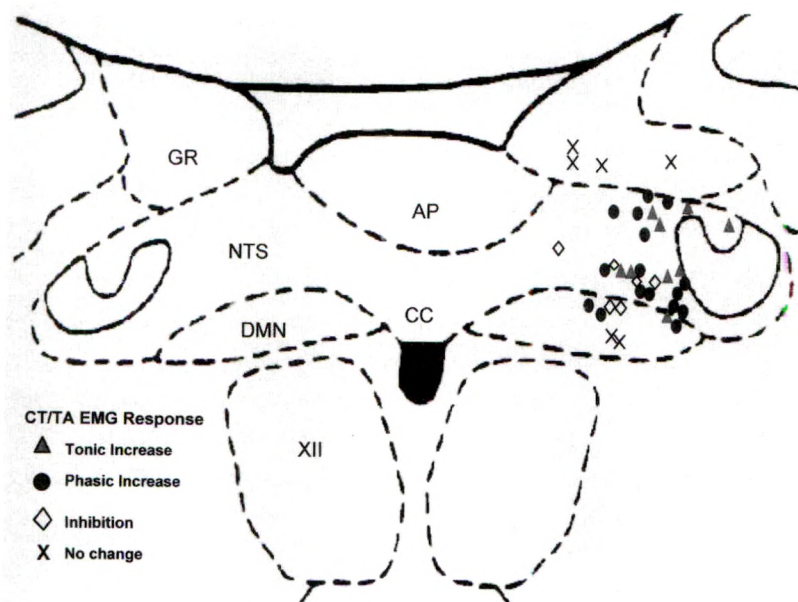


Fig 7. CT and/or TA EMG responses to SP injections at level of area postrema (AP), plotted on transverse schematic projection. Dye diffused into adjacent tissue in spherical pattern, but only central point of injection site is depicted. CT and/or TA EMG responses were variable, and clear correlation between responses and subnucleus of NTS injected was not evident. Tonic response was noted for injections into NTS, but not for injections into DMN or GR. Injections contained entirely within GR failed to elicit change in CT and/or TA EMG activity. Injections at ventral aspect of DMN similarly failed to elicit changes in CT or TA EMG activity, but those close to junction of DMN and NTS did alter CT and/or TA EMG activity. Responses cranial and caudal to AP are not depicted here. XII — cranial nerve XII; CC — central canal.

of their apnea. The duration of apnea was also analyzed for the subsets of animals whose halo of dye injection was contained entirely within the NTS, DMN, or GR without apparent overlap. Those animals that received injections into the NTS ($n = 15$) had significantly longer apnea than those animals that received injections into the DMN ($n = 5$) or GR ($n = 4$; 73.8 versus 3.2 versus 0.5 seconds, respectively; $p < .05$).

The severity of the laryngeal response and the duration of central apnea did not correlate directly or indirectly. Tonic contraction of the CT and TA muscles occurred in animals with long periods of central apnea (Fig 2), as well as in animals with brief periods of central apnea (Fig 3). As noted above, the responses of the CT and TA muscles and the DP were sometimes divergent. In some animals, cessation of DP EMG activity was accompanied by cessation of CT and TA EMG activity, whereas in other animals,

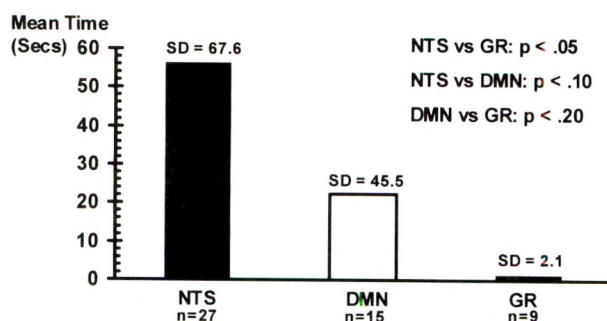


Fig 8. Comparison of durations of central apnea in seconds resulting from injections of SP into NTS, DMN, and GR. Duration of apnea after injection of NTS tended to be longer than that after injection of DMN, and was significantly longer than that after injection of GR. Duration of central apnea from NTS injection was underestimated in animals that required resuscitation.

it was accompanied by tonic activity of the muscles, as noted in the response tracings.

Cardiovascular Response. All animals experienced a decrease in BP and heart rate after SP injection. The decrease was significantly greater in animals that received injections into the NTS than in those that received injections into the GR or DMN ($p < .005$; Fig 9).

RESPONSE TO CONTROL INJECTIONS

Control injections of SP vehicle (20 nL; $n = 6$) and of artificial cerebrospinal fluid (20 to 80 nL; $n = 8$) into the NTS did not induce a significant change in DP activity, CT and TA EMG activity, BP ($p > .05$), or heart rate ($p > .05$).

DISCUSSION

The NTS is located in the medulla oblongata and

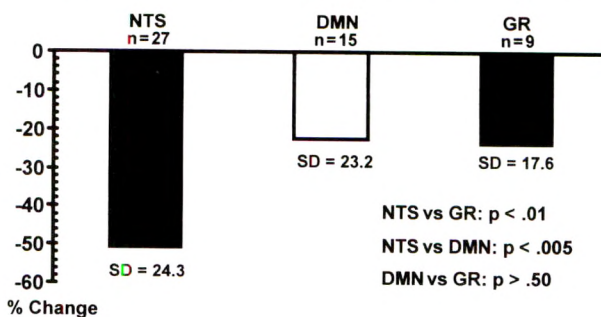


Fig 9. Comparison of percent changes in mean diastolic BP induced by SP injections into NTS, DMN, and GR. Decrease in BP was significantly greater for NTS injections. Mean baseline and mean nadir diastolic BPs for NTS, DMN, and GR were 113 and 56 mm Hg, 113 and 88 mm Hg, and 115 and 87 mm Hg, respectively. (See Materials and Methods section for formula calculating mean BP and percent change in BP.)

plays a critical role in maintaining homeostasis and in regulating visceral and autonomic activity. The NTS is a major site of termination of afferent fibers of cranial nerves V, VII, IX, and X.^{6,7} The NTS is a relay center that receives sensory information from these cranial nerves and sends premotor projections to other brain stem nuclei to evoke responses in the respiratory, gastrointestinal, and cardiovascular systems and the larynx. The laryngeal premotor neurons in the NTS project to motor neurons in the nucleus ambiguus that induce laryngeal motor activity.^{8,9}

The neurons of the NTS are topographically arranged in a cranial-caudal direction such that the regions related to gustatory function are located rostrally and the regions related to pharyngeal, laryngeal, respiratory, cardiovascular, and gastrointestinal activities are sequentially represented more caudally.^{6,10} The NTS is further subdivided into 9 subnuclei whose individual functions remain to be clearly elucidated. Future studies may delineate which subnucleus or subnuclei predominate in regulating laryngeal motor activity.

Although more than 25 neurotransmitters and neuropeptides have been identified in the NTS, SP is one of the neuropeptides found most frequently, and it is thought to be predominantly vagal in origin.^{11,12} Substance P is a member of the family of tachykinins, a group of biologically active peptides, and it mediates the transfer of sensory information from the periphery to the central nervous system.

Application of methylene blue and pontamine sky blue to the brain stem injection sites allowed us to determine the central point of injection postmortem. Unfortunately, the spherical diffusion pattern of the dye cannot be used to predict the diffusion pattern of SP or the control solutions, as each has a different hydrophilicity and interaction property with the tissue of the NTS. Additionally, only 10 nL of indicating dye was injected, while 20 nL of SP was injected. Despite these differences in methodology, we noted that all injections of SP in which the central portion was contained within the NTS elicited changes in the CT and TA EMG activity. Injections of SP in which the dye sphere was completely contained within the GR or DMN failed to elicit a change in laryngeal activity in 8 of 9 animals and produced only a brief period of silence in CT and TA EMG activity in the ninth animal, confirming that these nuclei are not integral to regulating SP-mediated CT and TA EMG activity. The DMN contains premotor neurons that predominantly innervate the smooth muscle of the lower esophageal sphincter and the stomach,¹³ while the GR receives afferent input from the abdom-

inal viscera and hindlimbs of rats.¹²

A change in CT and TA EMG activity was noted for injections of SP in which the central point was at the ventral aspect of the DMN and in which the halo of dye extended into the NTS. The changes paralleled those seen for injections into the NTS and are likely explained by diffusion or extension of the SP into the NTS.

We chose to monitor EMG activity of the CT and TA muscles of the larynx because these muscles are easily accessible in the small rat larynx. The CT and TA muscles actually play a more important role in tensing the vocal cords than in adducting them. Nevertheless, we have consistently observed in dogs and pigs that visible laryngospasm is accompanied by tonic EMG activity of both the TA and CT muscles.^{1,2,5} We believe that these muscles are activated in concert with the interarytenoid and lateral cricoarytenoid muscles — the primary adductor muscles of the larynx. In support of this belief, reflex laryngeal closure during Valsalva maneuvers in humans has shown that the TA muscle and the interarytenoid muscle are activated in concert with each other.¹⁴ Similarly, activity of the CT and TA muscles parallels that of the interarytenoid and lateral cricoarytenoid muscles during acts of phonation and swallowing.¹⁵ It is thus likely that the adductor and tensor muscles of the larynx act in concert with each other during periods of reflex laryngeal closure.

Activities of the laryngeal CT and TA muscles and the DP are closely linked during quiet respiration, but it is apparent from this study that the two systems can respond divergently to the same sensory stimulus. Injection of SP into the NTS consistently inhibited respiratory activity (absent DP EMG signal), while sometimes causing inhibition and at other times causing excitation of CT and TA EMG activity (Fig 4 versus Fig 2). The response patterns did not correlate with the order of injection or the side of injection. These divergent responses to sensory stimulation are in contrast to the coordinated, simultaneous activity of the CT, TA, and DP muscles observed in all animals during baseline recordings.

Other investigators have demonstrated that stimulation of the DMN caudal to the fourth ventricle causes relaxation of the lower esophageal sphincter, while stimulation rostrally causes contraction.¹⁶ The relatively large diffusion pattern of the dye prevented us from determining whether a similar rostral-caudal correlation exists for laryngeal activity.

The information in this study raises interesting clinical considerations with regard to intraoperative or postoperative "laryngospasm," a phenomenon well

recognized by surgeons and anesthetists. This phenomenon appears to occur in some patients who receive a sensory stimulus while under a light plane of anesthesia. The inciting stimulus may be clearly evident, such as abruptly moving a patient, or merely presumed, such as pooling of secretions on the laryngeal mucosa. The ensuing vocal cord adduction causes complete upper airway obstruction and eventual hypoxemia. Mask ventilation is impossible until the vocal cords spontaneously relax or are pharmacologically relaxed by administration of a neuromuscular blocking agent.

Our clinical experience suggests that a large number of patients receive sensory stimulation while emerging from general anesthesia, particularly after undergoing procedures on the upper airway, but only a small number of patients subsequently develop laryngospasm. This variable predilection parallels the variable response seen in the rats in this study. Whereas some animals developed life-threatening, prolonged activation of the CT and TA muscles in response to SP NTS injection, other animals developed only a phasic increase in adductor-tensor activity. Perhaps a sensory stimulus in humans under general anesthesia results in a release of SP from sensory nerves terminating in the NTS that evokes a spectrum of laryngeal responses ranging from brief inhibition of adductor-tensor activity to prolonged tonic activity.

We cannot exclude that variables in the experimental design accounted for the variable CT and TA EMG response patterns observed in the animals. The injections were not confined to a particular subnucleus of the NTS, and much diffusion of the SP to adjacent subnuclei did occur. Although any potential effect of ketamine and xylazine should have waned by the time the animals were ready for the brain stem injections, subtle variations in the plane of anesthesia maintained with α -chloralose may have affected laryngeal activity.

The tendency of laryngospasm and other life-threatening laryngeal reflex responses to occur far more frequently in pediatric patients than in adult patients may reflect developmental changes in sensitivity to SP in the central sensory pathways of the larynx. Histologic studies have shown greater SP im-

munoreactivity and SP receptors (NK1) in the NTS of young rats as compared to aged rats, and injection of SP results in greater changes in tidal volume and minute ventilation in younger animals.¹⁷ Indeed, we have observed that developing puppies are more prone to experience life-threatening laryngospasm induced by distal esophageal sensory nerve stimulation than are adult dogs.¹

Injection of SP into the NTS induced a significant decrease in systemic BP. This finding is in agreement with some studies¹⁸ but in contradistinction to other studies, which have shown no change in BP following SP injection.^{19,20} The reason for these divergent responses is unknown, but is perhaps related to variation in use of anesthesia or to subtle and unrecognized differences in the sites of injection. We did not use halothane, which has been found to modify central reflex activity arising from the NTS.²¹ Although apnea could have contributed to hypotension in some animals, there was no clear correlation between the two, and hypotension occurred in some animals that did not develop apnea.

Pretreatment of ferrets with an SP antagonist abolishes morphine-induced emesis; this finding suggests clinical implications for pharmacological blockade of the NK1 receptors.²² Such pharmacological blockade may ultimately also prove useful for management of pediatric patients prone to laryngospasm and of infants with life-threatening laryngeal reflex activity.

SUMMARY

1. Cricothyroid and TA EMG activity responses can be evoked in rats by injection of the sensory neurotransmitter SP into the NTS.
2. Injection of SP into the rat NTS evokes a variable response in laryngeal motor activity, ranging from inhibition (apnea) to tonic contraction (laryngospasm).
3. Although EMG activity of the DP and that of the CT and TA muscles are linked during quiet respiration, SP application to the NTS region sometimes causes divergent responses in the systems, ranging from inhibition to excitation.

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TREATMENT OF VOCAL FOLD SCARRING: RHEOLOGICAL AND HISTOLOGICAL MEASURES OF HOMOLOGOUS COLLAGEN MATRIX

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The current treatment options for dysphonia secondary to vocal fold scarring are limited. Few studies address changes in the lamina propria, which is critical to vocal fold biomechanical properties and voice production. Using rheological and histological measures of homologous collagen matrix (HCM)-injected vocal folds, we assessed HCM's potential for providing bulk and restoring biomechanical performance. Twenty rabbits underwent bilateral vocal fold scarring. After 10 weeks of scar maturation, the rabbits had unilateral injections of HCM or saline solution. Ten weeks after the injections, histological studies revealed well-defined collagen globules distributed throughout the lamina propria and underlying muscular tissue. Significantly more procollagen was observed in the HCM-treated group. No significant differences in elastic shear modulus or dynamic viscosity were found between the treatment groups. This study demonstrates that HCM is an inert, relatively stable injectate that may serve well for medialization but does not appear to improve the dynamic properties of the lamina propria.

KEY WORDS — collagen, dynamic viscosity, larynx, procollagen, rabbit, scar, shear modulus, vocal fold.

INTRODUCTION

Vocal fold scarring is a problem that results in dysphonia and can have a significant negative impact on quality of life. Various mechanisms of laryngeal injury can lead to vocal fold scarring, including trauma, phonosurgery, radiotherapy, inflammatory processes, and injury related to endotracheal intubation. Many substances and techniques have been devised to address this problem, ranging from synthetics to various bioimplantable materials.¹⁻³ Synthetic substances such as Teflon and gelatin have been used to achieve favorable short-term results,^{1,4,5} but their use has been plagued by long-term negative effects, including foreign body reaction, granuloma formation, and extrusion.⁶ More recently, biological implantables such as autologous fat and collagen have been used with promising results.^{2,3,7,8} Each material has unique handling properties, as well as distinct advantages and disadvantages.

Autologous fat is easily harvested from the abdomen and can be processed on the surgical field. The

short-term results for medialization, as well as treatment of mucosal injury, have been encouraging,^{8,9} but the long-term survival of fat grafts has been questioned.¹⁰ Bovine dermal (heterologous) collagen was developed as an early alternative to synthetic materials, but concern over immunologic reaction has limited its use. Autologous collagen harvested from the patient's abdominal skin avoids immunologic risk and promises greater longevity than fat.^{11,12} However, the current method for obtaining and processing this material is time-consuming and costly. Homologous collagen matrix (HCM) is a stable, nonimmunogenic substance with promising applications for treatment of vocal fold scarring (Collagenesis, Inc, Beverly, Massachusetts). Obtained from a cadaveric dermal source, it is processed to render it an acellular, purified, collagen matrix. It is easily stored and can be uniformly and precisely injected through a 27-gauge needle. The current study is designed to evaluate HCM as a novel injectate for the scarred vocal fold.

In the present study, we used a rabbit model that

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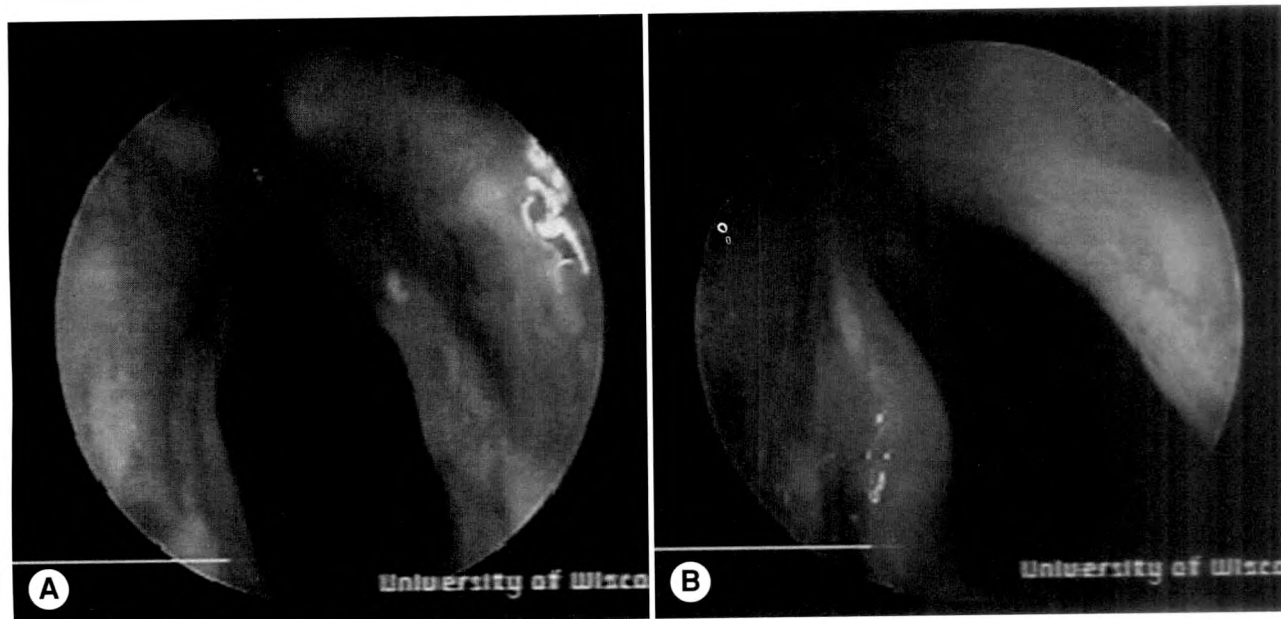


Fig 1. Examples of rabbit vocal folds **A)** after bilateral scarring procedures and **B)** immediately after injection of homologous collagen matrix (HCM) into left vocal fold.

in prior work was used to examine the scarred vocal fold relative to native vocal folds.¹³ Rabbit HCM was specially prepared with the protocol used to produce human HCM. To assess the *in vivo* performance of the HCM, we obtained data pertaining to the viscoelastic properties of HCM-injected scarred vocal folds. No study to date has assessed the *in vivo* biomechanical properties after treatment for scarring. To assess the fate of the injected HCM, we performed histological and immunohistochemical stains.

MATERIALS AND METHODS

HCM Processing and Production. Injectable collagen samples were prepared from New Zealand White rabbit dermis by Collagenesis, Inc, by a protocol similar to that used in the production of human Dermalogen, which is an injectable donor collagen implant. Briefly, this procedure involved soaking the rabbit pelt in 70% reagent alcohol for 10 minutes and then placing it in a sodium chloride solution (effectively aiding in removal of the epidermal layer of skin) for 24 hours at 4°C. The pelt was removed and washed 4 times with water. The fur and epidermis were scraped off to leave dermis, which was virally inactivated by soaking it in an alkaline solution (pH > 12) for 1 hour and washing it 3 times with neutral phosphate buffer followed by an acid soak (pH < 2.5) for 1 hour. The acid was neutralized, and the dermis was decanted and frozen in liquid nitrogen. The frozen dermis was milled into a granular powder (particle size > 500 µm) and resuspended in phosphate buffer pH 9. The dermal suspension was dispersed with the aid of an acylating agent by extrusion through a macerator. The macerated dermal dispersion was

centrifuged, and the supernatant was decanted. The top layer of the precipitate was removed and saved. The precipitate was resuspended in phosphate buffer and centrifuged again. The supernatant was decanted, and the top layer of the precipitate was removed and combined with the first saved precipitate. The mixed precipitate containing dispersed dermal matrix was suspended in 70% reagent alcohol overnight. The following day, the solution was centrifuged and the alcohol was decanted. The precipitate was washed 3 times with 0.004 mol/L phosphate buffer + 2% glycerol, with centrifuging after each wash. The final matrix dispersion was thoroughly mixed with a static mixer and then placed in a syringe. The final dispersion contained a suspension of intact collagen fibrils, elastic fibers, proteoglycans, and adhesive glycoproteins. The collagen concentration ranged from 15 to 30 mg/mL. The pH ranged from 7.0 to 7.6.

Surgical Scarring Technique. Twenty adult New Zealand White rabbits were anesthetized by means of an intramuscular dose of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg). Topical lidocaine spray was used to decrease laryngeal excitability before direct laryngoscopy with a neonatal laryngoscope (Pilling Co, Fort Washington, Pennsylvania). Before suspension of the laryngoscope, adequate exposure of the glottis was obtained. Two-millimeter microcup forceps (Microfrance, Montreal, Canada) were used to perform uniform bilateral vocal fold stripping under direct vision, yielding 40 scarred vocal folds.

Injection Technique. Ten weeks after scar formation (Fig 1A) by the above-described laryngoscopic

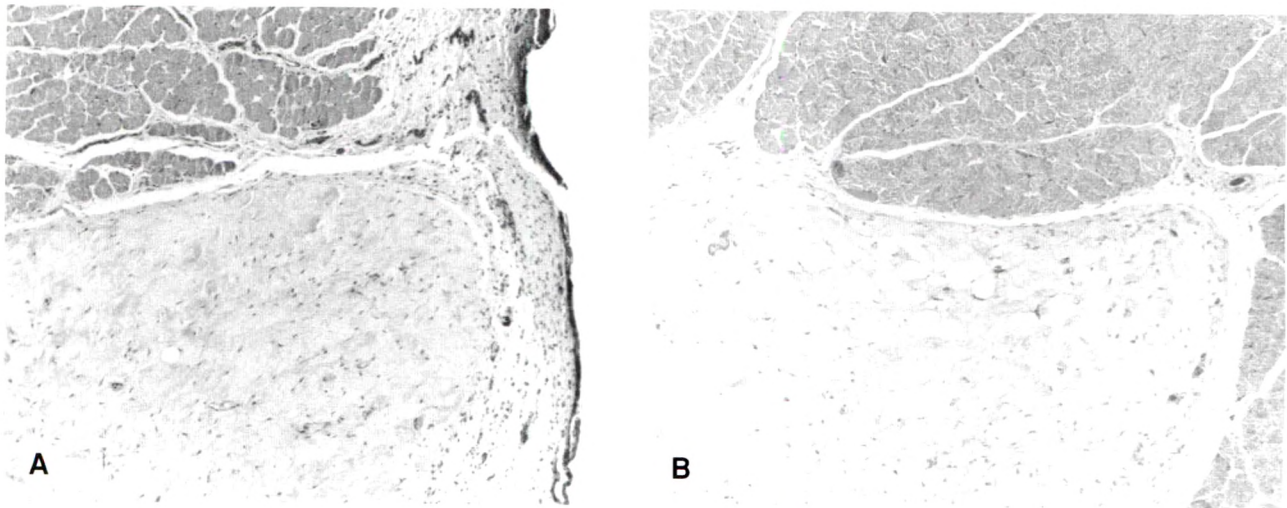


Fig 2. Trichrome-stained sections of HCM (original $\times 10$). **A)** In superficial and intermediate layers of vocal fold. **B)** In deep layer of vocal fold.

suspension technique, 10 rabbits underwent injection of 0.18 mL of HCM through a 27-gauge needle into the left vocal fold. The remaining 10 rabbits underwent “sham surgical” injection of 0.18 mL of sterile saline solution to the left side. Injections were made into the more superficial layers of the lamina propria. Figure 1B demonstrates the appearance of a vocal fold immediately following HCM injection. Visualization of the injected HCM demonstrated displacement of the medial edge of the vocal fold. Of note, 1 rabbit died of myocardial infarction 2 days after injection.

Harvesting Larynges. Ten weeks after injection, all rabbits were euthanized with an intravenous injection of Beuthanasia (0.22 mL) after induction of anesthesia as described above. The larynges were harvested immediately after death. The larynges to be used for rheological analysis were immediately frozen in liquid nitrogen and stored at -80°C ; the larynges to be used for histological analysis were placed in 10% formaldehyde.

Rheological Measurements of Viscoelastic Properties. The linear viscoelastic shear properties of various materials have been well studied by Chan and Titze.¹⁴⁻¹⁷ By this method, a rheometer (Bohlin CS-50 Rheometer; Bohlin Instruments Inc, Cranbury, New Jersey) was used to measure elastic shear modulus (G') and dynamic viscosity (η') as a function of oscillatory frequency (0.01 to 15 Hz). The above rheometer uses a parallel-plate configuration with a stationary lower plate offset by a 0.3-mm gap from a rotating upper plate. In this study, samples of lamina propria tissue were dissected from 18 vocal folds (6 saline-treated, 6 HCM-treated, and 6 with no treatment). Each sample placed between the plates was subjected to a sinusoidal shear stress at small ampli-

tude. A transducer measured the resultant shear strain, allowing for computations of elastic shear modulus (G') and dynamic viscosity (η') of the samples. Elastic shear modulus represents a measure of the material's stiffness in shear, and dynamic viscosity is the measure of a material's resistance to shear flow.

Histological Measurements. Twenty vocal folds removed from larynges after formaldehyde fixation were processed, paraffin-embedded, and cut into 5- μm -thick sections (Leica 2135 microtome, Leica, Boston, Massachusetts). The collagen content was assessed with a trichrome stain. Procollagen I was assessed by an immunohistochemical method with monoclonal antibodies (SO1.D8, Hybridoma Bank, Iowa City, Iowa). Rabbit mitral valve tissue served as the control. Image analysis was completed for procollagen I with a Nikon Eclipse E600 microscope (Nikon, Melville, New York), a Pixera color camera (Pixera, Los Gatos, California), and Metamorph Image Analysis Software (Universal Imaging, West Chester, Pennsylvania). The intensity of procollagen I was measured in the lamina propria at $20\times$ magnification; the measurement started at the superficial layer and progressed to thyroarytenoid muscle. Normalization of image intensity was completed for vocal folds of different thicknesses.

RESULTS

Histological Analysis. The HCM was clearly identified in all injected vocal folds via trichrome staining. Interestingly, HCM was found in all layers of the vocal fold in discrete globules (Fig 2). There was minimal to absent inflammatory reaction surrounding the HCM globules, with a paucity of identifiable lymphocytes. The HCM did not appear to incorporate into the surrounding tissue, regardless of in which layer it was found. Little to no cellularity was noted

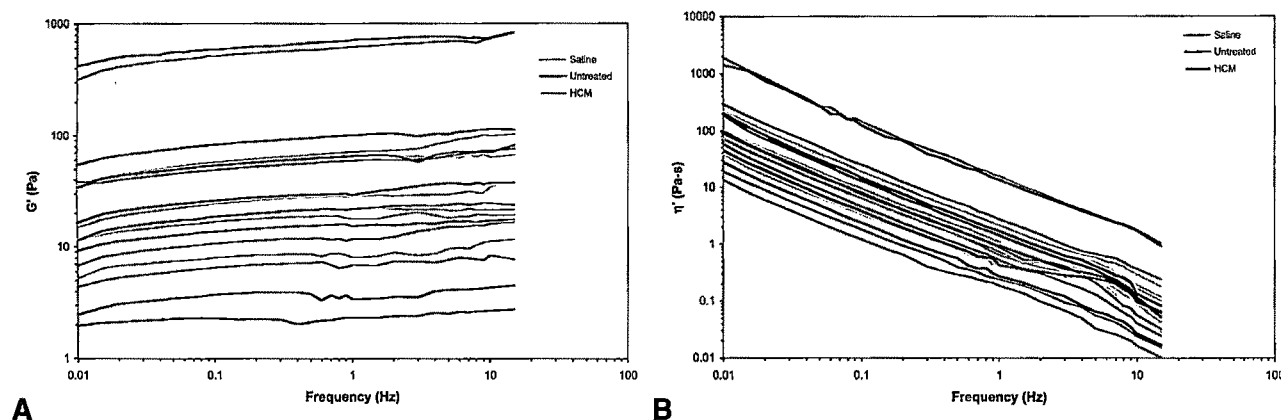


Fig 3. Graphs of **A)** elastic shear modulus (stiffness; G') and **B)** dynamic viscosity (η') of HCM-treated, saline-treated, and untreated groups, as function of oscillatory frequency of rheometer.

within the HCM injectate, indicating minimal in-growth of surrounding cells despite the matrix composition of the injectate.

The intensity level of procollagen I was measured in 5 HCM-treated and 5 saline-treated larynges. Procollagen I was found to be distributed throughout the superficial and intermediate layers of the lamina propria. The average intensity levels from these 2 groups were compared by use of a *t*-test from SAS Proc Univariate (SAS Institute Inc, Cary, North Carolina). There was a statistically significant difference between the groups ($p = .0153$), indicating an increase in procollagen I in the HCM-treated group.

Rheological Analysis. The data were analyzed with a multivariate repeated-measures analysis of covariance assuming a first-order autoregressive error structure within a subject. Estimation was performed with the SAS software procedure Proc Mixed (SAS Institute Inc). The independent variables were log frequency and treatment groups (untreated, saline-treated, and HCM-treated). Both elastic shear modulus and dynamic viscosity were first-log transformed to better meet the assumptions of analysis of covariance. Figure 3A shows the elastic shear modulus of tissue samples from the 3 treatment groups as a function of oscillation frequency on a log-log scale. Large inter-rabbit differences in tissue stiffness can be observed — as much as 2 orders of magnitude. For log elasticity, the difference between the data for the 3 groups when exponentiated indicated no significant difference ($p = .87$). Figure 3B shows the dynamic viscosity of the samples from the 3 groups as a function of frequency. Large inter-rabbit differences in tissue viscosity were again evident. For log viscosity, the difference between the group data when exponentiated also indicated no significant difference ($p = .90$).

DISCUSSION

There has been extensive work in the literature de-

voted to treating vocal fold scarring with various bio-implantable materials. Prior work has also addressed the viscoelastic differences between normal vocal fold tissue and scarred vocal folds.¹³ In choosing a material for vocal fold implantation or injection, the properties considered most often are ease of handling, precision of injection, immunogenicity of the material, and stability of the implant. Most important, the in vivo performance of an ideal substance should closely match the viscoelastic properties of the native vocal fold mucosa, and ideally should restore a normal mucosal wave. Work has been performed quantifying the in vitro dynamic viscosity and elastic shear modulus of many substances.¹⁴⁻¹⁷ However, the in vivo performance of bioinjectables has not been studied. In this study, we have measured the viscoelastic and histological properties of HCM, a novel bioinjectate, after injection into the scarred rabbit vocal fold.

The rabbit vocal fold has been studied and has an ultrastructure similar to that of the human vocal fold.^{13,18} Scarring induces changes in the ultrastructure of the lamina propria characterized by increased rates of procollagen production.¹³ Phonosurgical treatment methods have historically focused on addressing 2 aspects particular to the scarred vocal fold: medialization of the stiffened fold and restoration of the altered biomechanical properties of the lamina propria. Medialization is often seen as a secondary goal, addressing glottic insufficiency, with the primary objective being restoration of an adequate mucosal wave.

The histological results pertaining to collagen distribution revealed that the injected collagen matrix distributes randomly and widely within the various layers of the vocal fold. Although HCM is very easily injected through a 27-gauge needle, only a portion of the material remains in place in the target site, the superficial lamina propria. This variable distri-

bution is likely related to changes that have occurred in the vocal fold structure because of scarring, as well as the inaccuracies inherent in injecting an area as small as the rabbit vocal fold. (The thickness of the superficial lamina propria is approximately 1 mm.) Figure 2 reveals that the HCM is deposited in large, discrete globules that are easily differentiated from the native collagen.

Analysis of the HCM and the tissue immediately surrounding the globules reveals minimal influx of inflammatory cells. One would expect some reaction to the injection of a foreign substance in the vocal fold, and the typical scar remodeling process involves an influx of fibroblasts and macrophages. However, it is clear that the area around the HCM shows sparse inflammatory cells or other changes. This apparently low-level immunogenic reaction confirms the concept that an acellular, homologous tissue source offers distinct advantages over certain other bioimplant options.

Prior research has shown that procollagen formation increases with injury.¹⁹ Procollagen, secreted by fibroblasts into the extracellular matrix of the vocal fold, undergoes cleavage of the N terminal and C terminal segments, which eventually polymerize into collagen fibrils. The significantly higher procollagen quantities found in our study suggest that injection of HCM stimulates procollagen formation in the injured vocal fold. Fibroblast influx related solely to the physical injection cannot account for the increase, as the saline control group did not show an increase. These results suggest that the HCM-injected vocal fold undergoes more intense remodeling, but not at the expense of increased immunogenicity or inflammatory response. The procollagen results suggest that over time, as HCM is resorbed, newly formed collagen could provide volumetric filling within the layers of the vocal fold. This is a novel finding, and further study of this aspect of HCM injection should be investigated. In the future, studies observing longer postinjection intervals may elucidate the effects of the heightened remodeling response.

Previous rheological work established that the elastic shear modulus (stiffness) and dynamic viscosity are significantly higher in scarred vocal folds than in normal controls.¹³ In the current study, the viscoelastic results are characterized by wide inter-rabbit variation in the HCM group, with no significant statistical difference between the HCM and saline control groups. One explanation for the wide variability is that HCM ended up being distributed within so many different levels of the vocal fold, from the lamina propria to the underlying vocalis musculature. The heterogeneity of the HCM-injected vocal fold most likely led to a wide standard deviation of viscosities between samples. Saline solution, once injected, is likely quickly resorbed, causing little change in the vocal fold. This lack of change is reflected in the narrow variability between saline-injected samples. Future work determining whether the HCM could be more precisely delivered to the superficial lamina propria could elucidate the in vivo biomechanical effects of this material more completely. Additionally, studying a larger number of animals may increase the statistical power and could demonstrate clearer differences between treatment groups.

CONCLUSIONS

The current study demonstrates that HCM is a relatively stable, minimally immunogenic material that may be used to address the difficult problem of the scarred vocal fold. The histological appearance suggests that HCM elicits very little inflammatory response and may be a safer, longer-lasting solution than other bioimplantable materials. It is relatively easy to handle and inject, although, as with all other materials, there is variability to the final location of the injectate. New collagen synthesis is stimulated in response to injection of HCM, as evidenced by augmented procollagen formation. The HCM was not shown to improve the biomechanical properties of the scarred vocal fold. According to these findings, HCM may be more appropriately used to medialize a stiffened scarred vocal fold that is contributing to glottic insufficiency, rather than to restore optimal viscoelastic properties of a deficient lamina propria.

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COCHLEAR IMPLANTATION IN THE ELDERLY: RESULTS AND QUALITY-OF-LIFE ASSESSMENT

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To assess the efficacy, quality of life, and complication rate of cochlear implantation in patients over 60 years of age, we performed a retrospective chart review of 31 cochlear implant patients more than 60 years old at the time of surgery (mean, 70 years; range, 62 to 86 years). All patients had improvement in their audiological test results after operation. Twenty-eight patients (93%) are regular implant users at a median follow-up of 12 months. Major complications occurred in 2 patients (6%). We conclude that cochlear implantation in the elderly population has excellent results, with a complication rate similar to that in patients less than 60 years old, and yields an improved quality of life.

KEY WORDS — cochlear implant, elderly, quality of life.

INTRODUCTION

Although other factors that vary with age may play a role in some of the hearing loss in the elderly, it has been shown that age-related degenerative changes occur in the auditory pathways.¹⁻⁶ This evidence, along with the high cost of cochlear implantation, has brought into question the utility of cochlear implants in older people. Several studies have examined the role of cochlear implantation in the older population.⁷⁻¹¹ These have differed in their inclusion criteria of patients in the elderly group and their methods of assessing the benefits to the patient. One report⁷ has examined cochlear implantation in patients older than 55 years, 1 report⁸ in those older than 60 years, and 3 reports⁹⁻¹¹ in those 65 years and above. All of these studies have concluded that elderly patients have improved hearing results after implantation.

Horn et al⁹ used a questionnaire to evaluate the preoperative history, ease of use of the device, benefit to the patient's daily activities, and quality of life. Kelsall et al⁸ used an abbreviated version of the questionnaire developed by Horn et al to evaluate this group of patients. These studies have examined the quality-of-life issues in these patients and compared the results of audiological tests to those of a matched group of younger adult cochlear implant patients. No study has compared the quality of life of the elderly patients with cochlear implants to that of those below the age of 60 years. In our study, we set out to evaluate the role of cochlear implantation in the elderly by examining their audiological test results and their quality of life. To assess their quality of life, we used a modified version of the questionnaire of

Horn et al and compared the results with those of a group of patients under 60 years old with cochlear implantation matched for duration of hearing loss at our institution. We also tried to find out whether the type of cochlear implant affects the audiological results or the quality of life in elderly patients.

METHODS

Of the 130 patients who have undergone cochlear implantation at University Hospital in the past 13 years, 94 have been postlingually, profoundly hearing-impaired adults. For the definition of our elderly population, we chose patients older than 60 years, because 60 is an average of the previous reports. The average ($\pm$ SD) age at the time of implantation for the entire cohort was 52 ± 2.5 years (range, 18 to 86 years). At the time of implantation, 61 (65%) were between the ages of 18 and 59 years (average, 42 ± 2.5 years) and 33 (35%) were older than 60 years (average, 70 ± 2.5 years; range, 62 to 86 years). Of the 33 patients in the group older than 60 years of age, 25 received the Nucleus device and 8 had a Clarion implant placed.

Each patient in this study underwent extensive preoperative medical, audiological, and psychological testing to assess his or her candidacy for implantation. The preoperative and postoperative audiological evaluations included a cochlear implant testing battery. In addition to pure tone audiometry, speech recognition was assessed by the 4-choice spondee test, CID sentences, monosyllabic words, and NU-6 words. Phonemic perception was assessed with medial consonants and vowels. The CID sentences with

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Fig 1. Cochlear implant questionnaire.

1. How long have you had a hearing loss? _____ years _____ months?	
2. How long were you unable to use a regular telephone with a hearing aid before cochlear implantation? _____ years _____ months?	
3. What was the cause of your hearing loss? _____	
4. Do you have any hearing in the ear that was not implanted?	Yes/No
5. Do you use a hearing aid on the ear that was not implanted?	Yes/No
6. How long has it been since the date of your cochlear implantation _____ years _____ months?	
7. How old were you at the time of the cochlear implantation? _____ Years	
8. How long did you wear a hearing aid on the ear that was implanted before getting the implantation? _____ years _____ months?	
9. On the average, how many hours per day do you use your cochlear implant? _____ hours	
10. Do you have difficulty placing the headset or magnet, changing the batteries, or operating the speech processor?	Yes/No
11. Can you tell the difference between a human voice and an environmental sound?	Yes/No
12. Can you tell the difference between a man's voice and a woman's voice?	Yes/No
13. Can you understand some words without the use of lip reading?	Yes/No
14. On the telephone, can you recognize a	
Dial tone	Yes/No
Busy signal	Yes/No
Voice	Yes/No
15. Can you talk on the phone using a simple code such as "yes, yes" and "no"?	Yes/No
16. Have you increased your social activities since implantation?	Yes/No
17. Has the quality or loudness of your own voice improved since implantation?	Yes/No
18. In general, has your "quality of life" improved since implantation?	Yes/No
19. Has your confidence improved since implantation?	Yes/No
20. Please circle the word that best describes your use of the speech processor in the following conditions:	
At home:	
Excellent Very good Good Fair Poor No use	
At work:	
Excellent Very good Good Fair Poor No use	
During social activities:	
Excellent Very good Good Fair Poor No use	
In quiet:	
Excellent Very good Good Fair Poor No use	
In noise:	
Excellent Very good Good Fair Poor No use	
21. Would you recommend a cochlear implant to a friend who is severely hearing impaired?	Yes/No
22. Knowing what you know now, if you could start over again, would you choose to go through cochlear implantation?	Yes/No

and without lipreading were used to evaluate lipreading skills. Postoperative testing was performed every 3 months for the first year of follow-up, every 6 months for the second year of follow-up, and yearly thereafter. The preoperative psychological evaluation included neuropsychological consultation and administration of the Beck Depression Inventory (BDI). The BDI is a questionnaire that screens for depressive symptoms.¹²

A 22-item questionnaire was mailed to all 94 adult cochlear implant recipients (Fig 1). The survey is a modified version of the questionnaire first developed by Horn et al.⁹ The questionnaire was developed to assess preimplantation history and postimplantation implant use, communication, and quality of life. All questionnaires returned within 6 months were used. The returned questionnaires were then analyzed according to the aforementioned patient populations.

To compare the complication rates in the two patient populations, we divided the medical and surgi-

cal complications into major and minor. Major complications are defined as those necessitating rehospitalization for medical management, explantation of the device, surgical reexploration, or any degree of facial paralysis, permanent or temporary. Minor complications are those that required only local care and reprogramming of the speech processor.

Analysis for comparing preoperative and postoperative results was performed with a paired *t*-test. Student's *t*-test was used to compare the means between the elderly and nonelderly groups. A χ^2 test, and when appropriate, Fisher's exact test, was used for comparing proportions. All statistical tests were performed with SAS software (Statistical Analysis Systems, Raleigh, North Carolina).

RESULTS

Audiological. The mean postimplantation follow-up was 12 ± 4 months for patients more than 60 years old and 14 ± 3 months for patients 18 to 59 years of

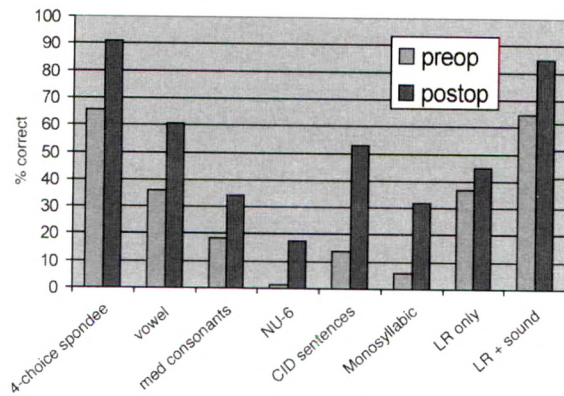


Fig 2. Preoperative and postoperative audiological testing in cochlear implant recipients more than 60 years old. LR — lipreading.

age. The mean pure tone average in the elderly population was 100 dB (range, 90 to 100 dB) before implantation, and improved to a mean of 37 dB (range, 5 to 60 dB) after implantation ($p < .0001$). The postoperative improvement of 4-choice spondee, vowel, medial consonant, NU-6 words, CID sentences, monosyllabic words, and lipreading with sound tests for the elderly population were all statistically significant and are presented in Fig 2. The mean postoperative improvement for the elderly group in 4-choice spondee scores was 26% ($p < .001$), in vowel scores was 31% ($p < .001$), in medial consonants was 15% ($p = .0148$), in NU-6 words was 17% ($p = .004$), in CID sentence scores was 40% ($p < .0001$), in monosyllabic words was 26% ($p < .0001$), and in lipreading with sound was 17.8% ($p = .042$). The average improvement in postoperative lipreading without sound was 3%, which was not statistically significant ($p = .64$).

A comparison of the preoperative and postoperative improvements in audiological testing between the elderly patients and those adult patients less than 60 years of age is presented in Table 1 and Fig 3. There were no statistically significant differences in improvements between the two patient populations

TABLE 1. MEAN PERCENTAGES IN IMPROVEMENT IN AUDIOLOGICAL TESTING IN ADULT COCHLEAR IMPLANT PATIENTS

	>60 Years Old	18-59 Years Old	p
Four-choice spondee	26	40	.0797
Vowel	31	40	.3118
Medial consonants	15	25	.1961
NU-6 words	17	20	.9788
CID sentences	40	49	.3431
Monosyllabic words	26	29	.6572
Lipreading only	3	5	.7681
Lipreading + sound	18	37	.0337

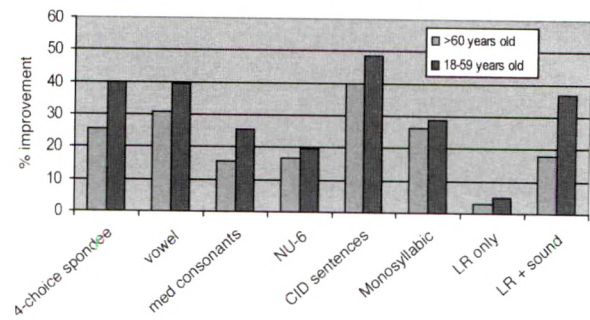


Fig 3. Postoperative improvement in percent on audiological testing in adult cochlear implant patients.

on any audiological test. The postoperative pure tone averages were 37 and 36 dB for patients more than 60 and those less than 60 years of age, respectively. This difference was not significant ($p = .6238$). In the elderly group, the type of cochlear implant, Nucleus versus Clarion, did not make a difference in the results in the entire audiometric battery. The preoperative neuropsychological consultation and BDI administration revealed no statistical difference in BDI scores in the two patient populations. The mean BDI scores were 8.6 (range, 2 to 26) for the elderly patients and 6.5 (range, 0 to 18) for those adult patients less than 60 ($p = .4517$). A BDI score of less than 14 is associated with no depressive symptoms, a score of 14 to 19 is associated with mild depression, and a score between 20 and 28 is associated with moderate depression.

Quality of Life. Ten of the 94 adult cochlear implant patients have relocated without a forwarding address. Of the remaining 84 patients who were mailed questionnaires, 61 (73%) returned them. The questionnaires were returned anonymously; however, the patients were identified with a small, discreet pre-printed number on the back of the questionnaire. The results were analyzed according to age at implantation. Thirty-two patients who were between the ages of 18 to 59 years at the time of implantation and 19 patients older than 60 years of age at the time of implantation returned the questionnaire. The mean duration of hearing impairment in the elderly and younger adult patient populations was 30 years (range, 1.5 to 74 years) and 23 years (range, 0.5 to 55 years), respectively ($p = .2670$). Before implantation, the duration of inability to use a telephone with a hearing aid (an indication of severe hearing loss) averaged 9.5 years (range, 0 to 42 years) in patients more than 60 years old and 4.0 years (range, 0 to 37 years) in patients 18 to 59 years old ($p = .3320$). Before implantation, hearing aids were used an average of 11 years (range, 0 to 56 years) in the elderly and 13 years (range, 0 to 36 years) in the younger adult patients ($p = .5409$). Of the older group, 12 patients had Nucleus implants and 7 patients had Clar-

	Age > 60 yrs	Age < 60 yrs	P value
1. How long have you had a hearing loss? Mean years (range)	29.5 (1.5-74)	23 (0.5-55)	0.2670 ^t
2. How long were you unable to use a telephone with a hearing aid before implantation? Mean years (range)	9.5 (0-42)	4 (0-37)	0.3320 ^t
3. Do you have any hearing in the ear that was not implanted? Yes: Number (%)	6 (25%)	7 (78%)	0.422 ^C
4. Do you use a hearing aid on the ear that was not implanted? Yes: Number (%)	8 (42%)	5 (16%)	0.036 ^C
5. How long has it been since the date of your cochlear implantation? Mean months (range)	41 (5-129)	48 (3-144)	0.2369 ^t
6. How old were you at the time of the cochlear implantation? Mean Years (range)	69 (60-86)	43 (25-59)	0.0001 ^t
7. How long did you wear a hearing aid on the ear that was implanted before getting the implantation? Mean years (range)	11 (0-56)	13 (0-36)	0.5409 ^t
8. On the average, how many hours per day do you use your cochlear implant? Mean hours (range)	12.5 (2-19)	14.5 (0-18)	0.0588 ^t
9. Do you have difficulty placing the headset or magnet, changing the batteries, or operating the speech processor? Yes: Number (%)	2 (10%)	2 (6%)	0.623 ^F
10. Can you tell the difference between a human voice and an environmental sound? Yes: Number (%)	18 (95%)	31 (97%)	1.000 ^F
11. Can you tell the difference between a man's voice and a woman's voice? Yes: Number (%)	16 (94%)	24 (77%)	0.230 ^F
12. Can you understand some words without the use of lip reading? Yes: Number (%)	17 (90%)	30 (94%)	0.623 ^F
13. On the telephone, can you recognize a			
Dial tone? Yes: Number (%)	14 (78%)	31 (97%)	0.050 ^F
Busy signal? Yes: Number (%)	12 (71%)	31 (97%)	0.015 ^F
Voice? Yes: Number (%)	9 (56%)	29 (91%)	0.010 ^C
14. Can you talk on the phone using a simple code such as "yes, yes" and "no"? Yes: Number (%)	10 (58%)	26 (84%)	0.055 ^C
15. Have you increased your social activities since implantation? Yes: Number (%)	12 (71%)	24 (75%)	0.739 ^C
16. Has the quality or loudness of your own voice improved since implantation? Yes: Number (%)	13 (72%)	28 (88%)	0.253 ^F
17. In general, has your "quality of life" improved since implantation? Yes: Number (%)	17 (94%)	30 (94%)	1.000 ^F
18. Has your confidence improved since implantation? Yes: Number (%)	15 (94%)	28 (90%)	1.000 ^F
19. Please circle the word that best describes your use of the speech processor in the following conditions:			
At home:			0.563 ^C
Excellent	5 (29%)	11 (34%)	
Very good	5 (29%)	12 (38%)	
Good	5 (29%)	5 (16%)	
Fair	1 (5%)	3 (9%)	
Poor	0 (0%)	1 (3%)	
No use	1 (5%)	0 (0%)	
At work:			0.344 ^C
Excellent	1 (10%)	6 (26%)	
Very good	1 (10%)	6 (26%)	
Good	4 (40%)	6 (26%)	
Fair	2 (20%)	2 (9%)	
Poor	0 (0%)	2 (9%)	
No use	2 (20%)	1 (4%)	
During social activities:			0.658 ^C
Excellent	1 (6%)	5 (16%)	
Very good	4 (24%)	7 (22%)	
Good	3 (18%)	8 (25%)	
Fair	7 (41%)	7 (22%)	
Poor	1 (6%)	4 (13%)	
No use	1 (6%)	1 (3%)	
In quiet:			0.260 ^C
Excellent	6 (35%)	17 (53%)	
Very good	5 (29%)	9 (28%)	
Good	4 (18%)	4 (13%)	
Fair	3 (12%)	2 (6%)	
Poor	0 (0%)	0 (0%)	
No use	1 (6%)	0 (0%)	
In noise:			0.581 ^C
Excellent	0 (0%)	2 (6%)	
Very good	3 (19%)	7 (22%)	
Good	1 (6%)	7 (22%)	
Fair	4 (25%)	5 (16%)	
Poor	6 (38%)	8 (25%)	
No use	2 (13%)	3 (9%)	
20. Would you recommend a cochlear implant to a friend who is severely hearing impaired? Yes: Number (%)	18 (94%)	32 (100%)	0.360 ^F
21. Knowing what you know now, if you could start over again, would you choose to go through cochlear implantation? Yes: Number (%)	18 (94%)	31 (97%)	1.000 ^F

^tdenotes t-test p value; ^Cdenotes Chi Squared test p value; ^Fdenotes Fischer's Exact test p value.

Fig 4. Cochlear implant questionnaire results.

ion devices. The results of the questionnaire can be seen in Fig 4. The only statistically significant differences between the elderly group and the younger adult population on the questionnaire were in recog-

nizing a busy signal and recognizing a voice. The quality-of-life assessment based on implant type, Nucleus versus Clarion, revealed no difference in outcome in the elderly group.

TABLE 2. MEDICAL AND SURGICAL COMPLICATIONS

	>60 Years Old (N = 33)		18-59 Years Old (N = 61)	
	No.	%	No.	%
Major				
Infection, hospitalization, intravenous antibiotics	0		3	4
Extrusion of electrode, explantation	0		2	3
Device failure	0		1	2
Perilymphatic fistula	1	3	0	
Infection, explantation	0		1	2
Temporary facial paralysis	1	3	0	
Total	2	6	7	11
Minor				
Facial stimulation, reprogrammed	4	12	7	11
Infection, oral antibiotics	4	12	5	8
Hypersensitivity to epinephrine	0		1	2
Total	8	24	13	21

Complications. There were no life-threatening complications in either patient population of adult cochlear implant recipients. There were 30 (32%) overall complications: 9 (10%) were major and 21 (22%) were minor. The medical and surgical complications are presented in Table 2. There was no statistical difference in the occurrence of major or minor complications between the elderly and younger adult patient populations. There was also no difference in the number of complications between the patients who received a Clarion implant and those who received a Nucleus device.

DISCUSSION

The results of this study suggest that cochlear implantation in the elderly provides improvements in audiological results and subjective quality of life similar to those in younger adult cochlear implant recipients. Further, there appears to be no increased morbidity associated with cochlear implant surgery in the elderly population. Previous studies have found similar results in regard to subjective improvements in quality of life in elderly cochlear implant recipients.⁷⁻¹¹ However, no study has compared quality-of-life issues of elderly patients to those of patients below 60 years of age. In doing such a comparison, we were able to more clearly define the benefits of cochlear implantation in the elderly.

In addition to age-related degenerative changes that occur in the auditory pathways,¹⁻⁶ recent evidence suggests that central auditory nuclei, cortices, and neurons undergo degenerative changes.⁴ These changes are proportional to the duration of profound hearing

impairment.⁴ Therefore, in elderly patients who, on average, experience longer periods of deafness, the benefit of a cochlear implant has been questioned. Further, it has been shown that the peripheral auditory system undergoes age-related degenerative changes.^{5,6} The combination of central and peripheral degenerative changes in the auditory system explains why simple amplification with hearing aids fails as the hearing impairment progresses. It also provides a simple explanation of how a cochlear implant, by bypassing the cochlea, benefits profoundly hearing-impaired patients.

The elderly patients had improvements in speech and phonemic recognition after implantation that were similar to those of younger adult patients. All audiological improvements made by the elderly patients were statistically significant, with the exception of medial consonants and lipreading without sound. The absence of significant improvement in lipreading without sound can be explained by the fact that it is not a test of a patient's ability to hear. Therefore, the improvement in lipreading with sound (17%) as compared to without sound (3%) exemplifies the benefit of cochlear implantation in the elderly. Further, although the percent improvement in medial consonants is not significant in the elderly, neither is a comparison of the improvements made between the elderly (15%) and the younger adult (25%) patients. In fact, when the two groups' audiological improvements were compared against each other, no statistically significant difference existed, so we conclude that cochlear implantation benefited both patient populations similarly.

Stronger evidence supporting cochlear implantation in the elderly is the subjective improvement in quality of life of the great majority of elderly patients. When compared to younger adult patients, they showed no difference in hours per day of implant usage, ability to discriminate environmental sounds from human voices, or ability to communicate via the telephone. The majority of elderly patients reported improved social life, confidence, and overall quality of life after implantation. When asked whether they would recommend cochlear implantation to a friend or opt to pursue cochlear implantation again, all but 1 patient (94%) answered positively. That patient commented on her questionnaire that if she had not had a complication, she would recommend it to a friend. Although these results are similar to those of previous reports,⁷⁻⁹ no previous study compared the subjective assessments of quality of life between elderly and younger adult populations. We have shown that cochlear implantation has allowed our patients to live more fulfilling and interactive lives.

This is the first report assessing the quality of life and audiological test outcomes in an elderly group on the basis of the type of implant. The 2 devices currently in use in the United States, the Nucleus device and that of Advanced Bionics, appear to be equivalent in the patients' perception of improvement in quality of life. Also, when the audiological test results are compared, there appears to be no difference in the outcome between the 2 devices.

Increased risks associated with surgical procedures in the elderly were not a factor in this study. The per-

centage of major surgical or medical complications in elderly cochlear implant recipients in this study was 6%, a value similar to that reported by Cohen et al¹³ for all adult cochlear implant patients. One elderly patient suffered temporary facial paresis — an incidence of 1%, which was similar to that of other studies.¹³ Of note, none of the patients in the elderly group had cardiovascular, respiratory, or central nervous system complications. Overall, the implantation surgery in the elderly was well tolerated, and the complication rates (minor and major) did not differ from those of younger patients.

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IMPACT OF VOCAL CORD PARALYSIS ON CRICOARYTENOID JOINT

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To demonstrate structural changes in the cricoarytenoid joint after recurrent laryngeal nerve paralysis, we performed a laboratory investigation of fixed arytenoid cartilages from adult humans obtained during laser surgical arytenoidectomy in cases of bilateral vocal fold paralysis, analyzing the articular cartilage, the joint capsule, and the attached laryngeal musculature. Ten arytenoid cartilages from adult humans were studied by means of histology, as well as scanning and transmission electron microscopy. After long-standing denervation (>6 months), all arytenoid cartilages showed degenerative changes in their joint surface structure at various levels of intensity. The articular surface revealed fibrillation in some places, demasking of collagen fibrils next to the joint surface, and formation of chondrocyte clusters near the joint surface. All specimens also showed muscle atrophy. We conclude that long-standing recurrent laryngeal nerve paralysis does not result in ankylosis of the cricoarytenoid joint, as assumed, but the articular cartilage undergoes structural changes comparable to those in osteoarthritis. Structural changes in the articular cartilage and in the surrounding musculature hamper efforts at joint function recovery, as do procedures aiming solely at either medialization or lateralization of the vocal fold.

KEY WORDS — cricoarytenoid joint, glottis, osteoarthritis, recurrent laryngeal nerve paralysis.

INTRODUCTION

Disturbances of vocal fold mobility following thyroid gland surgery and endotracheal intubation maneuvers are not uncommon. These complications include impaired movement of one or both vocal folds due to cricoarytenoid joint (CAJ) dysfunction and/or recurrent laryngeal nerve damage, and may be characterized by hoarseness or stridor.

The human CAJ can be involved in a number of different pathological conditions. Trauma,¹⁻³ direct bacterial infections of the larynx,⁴ and joint diseases such as arthritis,⁵ osteoarthritis,^{6,7} rheumatoid arthritis,⁸ gout,⁹ or Tietze's syndrome¹⁰ have all been reported to occur in the CAJ. Interestingly, and in contrast to the limb joints, publications elucidating the influence of recurrent nerve damage on the morphology of the CAJ have, to date, been restricted to some histologic investigations.

Anatomically, the CAJ is a diarthrodial joint supported by a wide joint capsule lined with synovium. The capsule is strengthened posteriorly by the cricoarytenoid ligament. The structural arrangement of the joint allows 2 principal types of motion: a rocking or rotating movement around the axis of the joint, and a linear glide parallel to this axis.¹¹ The function is to control the abduction, adduction, and length of the vocal cords, thereby facilitating respiration, protecting the airway, and permitting phonation.

The present investigation analyzes the influence of recent and long-standing recurrent laryngeal nerve

paralysis (RLNP) on the structure of the CAJ and correlates the findings with laryngeal anatomy. Moreover, the findings are discussed in relation to resulting biomechanical function, compared with osteoarthritic changes in the CAJs, and related to the implementation of thyroplasty procedures.

MATERIALS AND METHODS

Arytenoid cartilages obtained from 10 patients who underwent an arytenoidectomy because of paralysis of the last remaining recurrent laryngeal nerve or of both recurrent laryngeal nerves (duration 6 weeks to 44 years) were chosen for this study. The cartilages were dissected by laser surgery in the ENT Department, Friedrich Schiller University, Jena, Germany. All of the arytenoid cartilages except for 1 were split into 2 halves after dissection.

The age, sex, and duration of RLNP of the 10 patients are shown in Table 1. The patients selected were free from recent trauma, neoplasm, and systemic inflammatory conditions and had not been subjected to radiotherapy. All specimens were associated with bilateral vocal cord paralysis. The duration of RLNP up to 6 months was classified as "recent," and longer than 6 months as "long."

A control series of 52 CAJs (30 from men and 22 from women; 20 days to 88 years of age) obtained from 26 body donors from the Department of Anatomy, Christian Albrecht University, Kiel, Germany, was used to differentiate possible morphological changes in the articular cartilage and the muscula-

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TABLE 1. LARYNGEAL FINDINGS IN PATIENTS WITH RECURRENT LARYNGEAL NERVE PARALYSIS

Case	Age (y)	Sex	Side	Duration of Paralysis	Special Features
1	69	M	R	9 mo	
2	45	F	R	13 mo	Two previous operations on right vocal ligament
3	74	F	R	44 y	
4	71	F	R	12 mo	Clinically, joint cavity partly filled with connective tissue
5	49	F	L	30 y	
6	78	F	L	6 wk	
7	74	F	R	19 mo	Initial laterofixation of arytenoid cartilage
8	69	F	R	30 mo	
9	55	F	R	18 mo	
10	69	F	R	27 mo	

In every case, recurrent laryngeal nerve paralysis was bilateral and due to strumectomy.

ture surrounding the CAJ. All specimens had been investigated in 2 previous studies^{2,12} describing the composition of the extracellular matrix in the articular cartilage of the CAJ and the morphology of its joint capsule and the joint-related structures.

Light Microscopy. After fixation in a 4% formalin solution and decalcification in 20% ethylenediamine-tetraacetic acid, 9 arytenoid halves (1 from a man and 8 from women; 45 to 78 years of age; Table 2) were embedded in paraffin and sectioned in a frontal or sagittal plane. Sections 7 µm thick were stained with toluidine blue at pH 1, azan, and resorcin-fuchsin-thiazine-picric acid and by the methods of Gomori and Goldner.¹³

Transmission Electron Microscopy. Transmission electron microscopy was performed on 2 arytenoid halves (from 2 women, 71 and 74 years of age; Table 2). Fixation in 3.5% glutaraldehyde was followed by decalcification, and the specimens were embedded in araldite. Semithin sections were prepared for light microscopy, and thin sections for electron microscopy. After contrast enhancement of the thin sections with uranyl acetate and lead citrate, examination was carried out with a Zeiss TEM 902 electron microscope.

Scanning Electron Microscopy. Scanning electron microscopic investigations were performed on 5 arytenoid halves and 1 whole arytenoid cartilage (1 from a man and 7 from women; 45 to 87 years of age; Table 2). After fixation in 2.5% glutaraldehyde and impregnation in 2.5% tannic acid, the preparations were counterfixed in 2% osmium tetroxide and dried in a critical point dryer. Finally, all specimens were

TABLE 2. INVESTIGATIVE METHOD APPLIED TO EACH ARYTENOID CARTILAGE

Case	Medial Half	Lateral Half
1	LM	SEM
2	LM	SEM
3	SEM	SEM
4	LM	TEM
5	LM	SEM
6	LM	SEM
7	LM	TEM
8	LM	SEM
9	LM	SEM
10	LM	SEM

LM—light microscopy, SEM—scanning electron microscopy, TEM—transmission electron microscopy.

coated with gold and their articular surfaces were analyzed with a Philips scanning electron microscope.

RESULTS

The duration of RLNP ranged from 6 weeks to 44 years (Table 1). All cases except 1 were of greater than 9 months' duration, and 2 cases were of 30 years and 44 years.

Light Microscopy. In the 6-week specimen, the arytenoid cartilage was histologically normal in terms of the integrity of the cartilaginous surface. Histologic examination of the arytenoid cartilage of the specimens ranging from 9 to 30 months of RLNP showed changes in the superficial cartilage layer with formation of chondrocyte clusters (Fig 1), a decrease of the total cell content in the upper cartilage layer with a clear demarcation line to the underlying "normal" cartilage (Fig 1), and a demasking of collagen fibrils in the surrounding extracellular matrix.

Additionally, all specimens that had between 9 and 30 months of RLNP revealed a surface roughening of the arytenoid cartilage surface. The arytenoid cartilage of the patient with a history of 30 years of RLNP demonstrated a fibrous coating, in some areas covering the underlying cartilage (Fig 2), and in other areas a pronounced surface roughening of the articular surface was visible.

No formation of fissures was found in the articular surface. There was atrophy of the laryngeal musculature (Fig 3) adhering to the excised arytenoid cartilages in all specimens except the one with a history of 6 weeks of RLNP.

Transmission Electron Microscopy. The 2 cases examined by means of transmission electron microscopy were from persons with histories of 1 year and 19 months of RLNP. Both cases revealed chondrocytes in clusters near the articular surface of the ary-

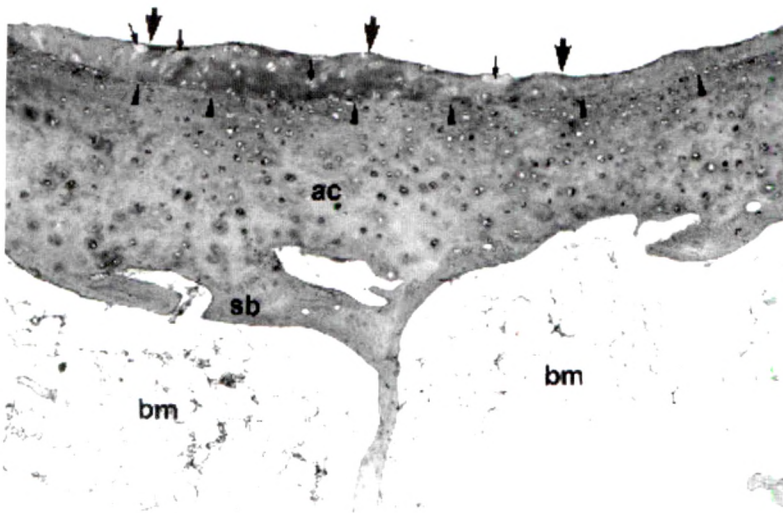


Fig 1. Frontal section through arytenoid cartilage (45-year-old woman with 13 months of recurrent laryngeal nerve paralysis [RLNP]) shows changes in superficial cartilage layer with formation of chondrocyte clusters (small arrows) and decreased total cell content in upper cartilage layer with clear demarcation line (arrowheads) to underlying articular cartilage (ac; azan, original $\times 90$). Large arrows — articular surface, sb — subchondral bone, bm — bone marrow.

tenoid cartilage, with rarefaction of typical cell organelles such as rough endoplasmic reticulum and mitochondria. The cytoplasm of these cells contained many large vacuoles, many lipid droplets, large Golgi complexes, and a relatively smooth and unscalloped

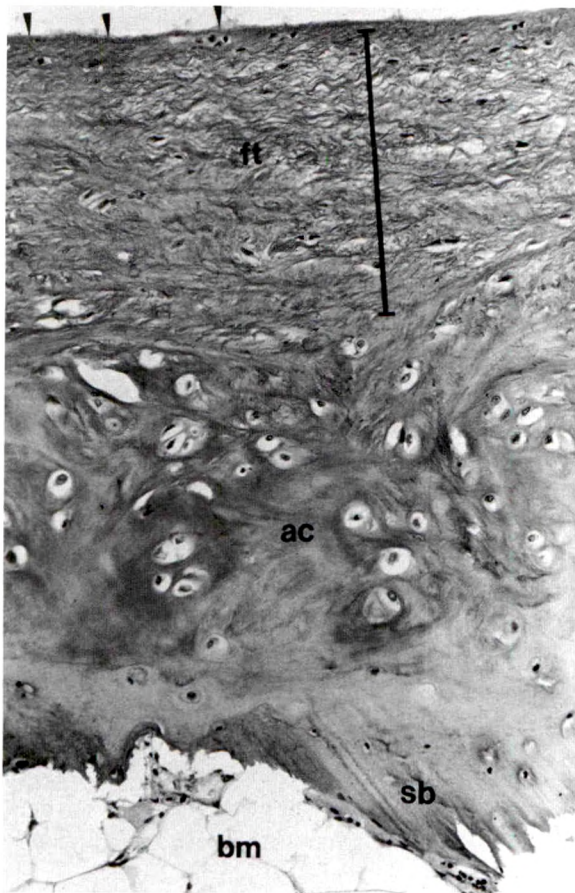


Fig 2. Frontal section through arytenoid cartilage (49-year-old woman with 30 years of RLNP) reveals coating of articular cartilage (ac) with fibrous tissue (ft; toluidine blue, original $\times 181$). Bar — extension of fibrous tissue, arrowheads — articular surface, sb — subchondral bone, bm — bone marrow.

surface membrane. The surrounding extracellular matrix of the clusters was characterized by inclusion of vesicles and granulations of various sizes and forms (Fig 4).

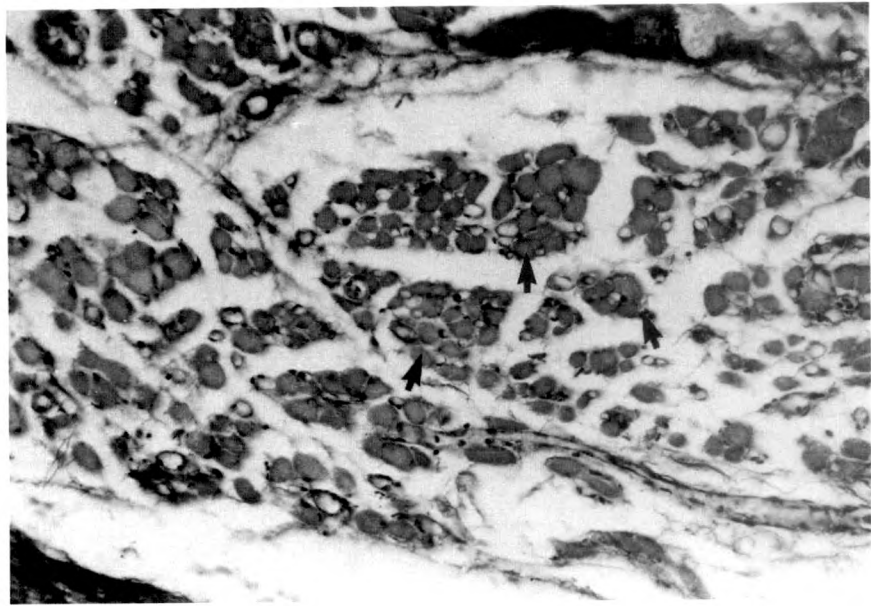
Scanning Electron Microscopy. Scanning electron microscopy revealed alterations of the articulating arytenoid facet halves at various levels of intensity. These levels were related to the duration of RLNP, with the fewest changes after 9 months of RLNP and the most changes after 44 years of RLNP. On the other hand, the arytenoid cartilage of the patient with a 6-week history of RLNP showed a smooth and symmetric articular surface without any alterations. All other arytenoid facet halves were characterized by a roughening of the articular surface, showing a cobblestone appearance (Fig 5). The 2 arytenoid cartilages with very long-standing paralysis (30 and 44 years) showed a fibrous coating in some areas of the articular surface and other areas with extensive surface roughening (Fig 6).

Controls. All findings in this study revealed age-related similar deviations from the healthy morphology of the articular cartilage and the structures surrounding the joints, as described in 2 previous studies,^{2,12} without any history of paralysis of the vocal folds.

DISCUSSION

In our study, we used light and electron microscopy to examine the articular surface of the arytenoid cartilage and the musculature near the CAJ in recent and long-standing cases of RLNP. Specifically, we studied potential structural alterations in the superficial and deeper layers of the articular cartilage, as well as in the musculature attached to the arytenoid cartilage. We found clear changes in cases of long-standing RLNP comparable to those of osteoarthritis in the arytenoid cartilage, as well as signs of mus-

Fig 3. Frontal section through laryngeal musculature adhering to excised arytenoid cartilage (69-year-old man with 9 months of RLNP; resorcin-fuchsin-thiazine-picric acid, original $\times 362$). Muscle fibers (arrows) are surrounded by fatty tissue, which has been dissolved by paraffin embedding and looks like hollow spaces.



cular atrophy.

Vocal fold immobility is a common problem in otorhinolaryngology practice. In cases of RLNP, a long period of uncertainty about recovery of the nerve function must be bridged over, as recovery of the damaged nerve can take as long as 390 days.¹⁴ Consequences of long-standing RLNP affecting the mobility of the CAJ were assumed and related to findings

in the limb joints, in which early and late changes of the articular cartilage, the joint capsule, and the surrounding musculature after immobilization are well known¹⁵ and frequently result in total immobilization of the joints involved unless continuous aggres-

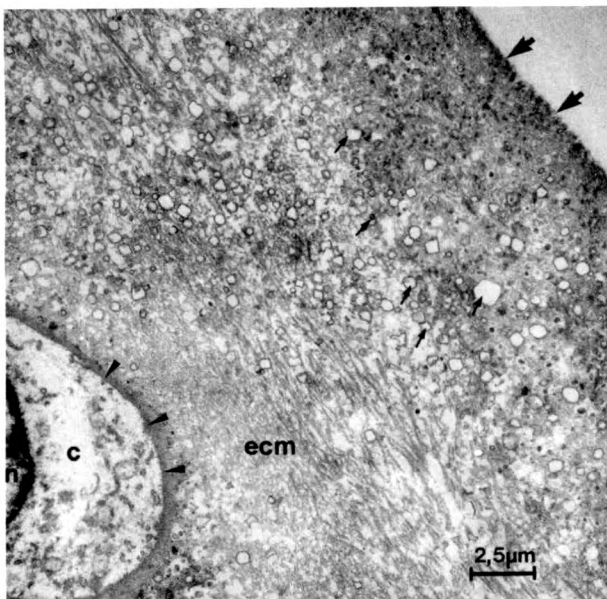


Fig 4. Transmission electron microscopy image of articular surface layer of arytenoid cartilage (74-year-old woman with 19 months of RLNP) shows part of chondrocyte (c) with rarification of typical cell organelles and relatively smooth and unscalloped surface membrane (arrowheads). Surrounding extracellular matrix (ecm) is characterized by inclusion of vesicles and granulations (small arrows) of different sizes and forms. Large arrows — articular surface, n — nucleus of chondrocyte.



Fig 5. Scanning electron microscopy image of articular surface of arytenoid cartilage (69-year-old man with 9 months of RLNP) shows roughening with cobblestone-like appearance.

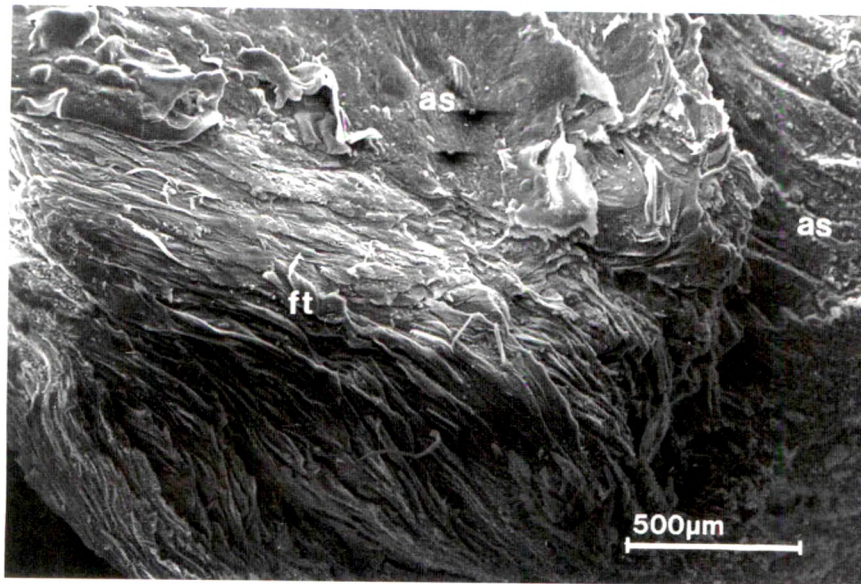


Fig 6. Scanning electron microscopy image of articular surface of arytenoid cartilage (74-year-old woman with 44 years of RLNP) shows, in some areas, fibrous tissue (ft) and, in other areas, roughened articular surface (as). Clinically, arytenoid joint surface was partly connected to cricoid joint surface.

sive physical therapy is administered. From the clinical impression of a fixed arytenoid cartilage, it was concluded that long-standing RLNP leads to ankylosis of the CAJ.^{14,16-18}

Messerklinger and Probst¹⁷ described fibrous obliteration of the joint space in 3 CAJs after RLNP. However, the period of 10 weeks after which fibrous ankylosis of the joint space was observed in their case 1 seems rather short for membrane formation without any signs of inflammation. We suggest that the authors misinterpreted large synovial folds of the joint capsule projecting intensively into the joint space. Such synovial folds, as recently described, can often be so large that they fill out the whole joint cavity in

histologic sections.^{2,3}

Using an experimental rabbit model in which the vagal nerves were cut through, Langnickel¹⁴ demonstrated beginning ankylosis of the CAJ after 5 months of denervation and complete ankylosis in nearly all animals after 12 months. However, caution is advised when extrapolating animal experimental data to humans, since the structure and function of the larynx vary widely in different species.

In contrast to these findings, Elies and Pusalkar,¹⁹ as well as Gacek and Gacek,²⁰ found on histologic investigation that there was no evidence of CAJ ankylosis, not even after 37 years of RLNP, and they concluded that the absence of CAJ ankylosis permits the possibility of complete recovery of joint function, in view of the integrity of the cartilaginous joint surfaces.

Our study confirms that there is no CAJ ankylosis, not even after 44 years. However, in contrast to the findings of Elies and Pusalkar,¹⁹ as well as Gacek and Gacek,²⁰ our findings reveal that there are clear structural alterations in the articular cartilage of the arytenoid, as well as in the surrounding musculature, in cases of long-standing RLNP (over 6 months' duration). These changes are comparable to osteoarthritic alterations of the CAJ as recently described.^{6,7}

Our results also confirm the finding that muscle atrophy of the laryngeal musculature does occur.²⁰⁻²² However, in contrast to Kano et al,²² who stated that muscle atrophy does not occur for at least 1 year after denervation, our results show muscle atrophy in a patient after as little as 9 months of RLNP (Fig 3).

One is led to question the reason for the discrepancy between limb joint ankylosis and CAJ movement after long-standing denervation. Two explana-

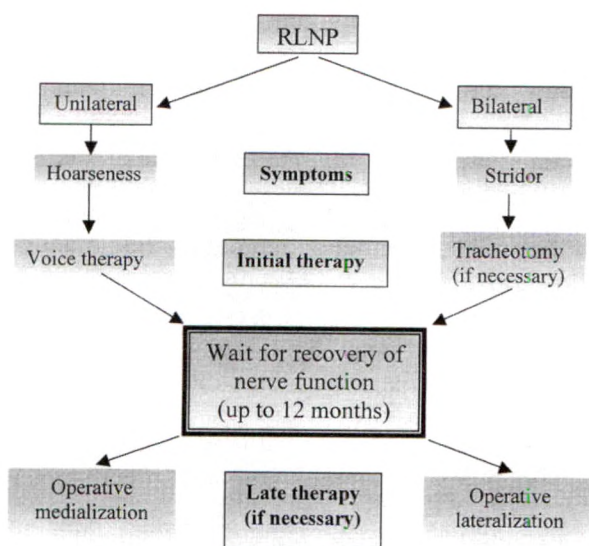


Fig 7. Timetable of therapeutic steps in management of unilateral and bilateral RLNP. There is problematically long immobilization period of cricoarytenoid joint until nerve recovery or larynx surgery.

tions are possible from an anatomic point of view. First, the CAJ is subjected to continuous passive movement during swallowing. Second, significant neural connections between the recurrent laryngeal nerve and the superior laryngeal nerve^{23,24} lead to small residual contractions of the laryngeal musculature as permitted by the still-functioning superior laryngeal nerve after RLNP.

On the basis of the irreversibility of most thyroplasty surgical interventions — indicated to relieve breathing or phonation disturbances due to RLNP — standard procedure is to wait roughly 1 year before surgical intervention (Fig 7). This is the reason for the problematic length of the immobilization period for the CAJ and inner laryngeal muscles between the manifestation of paralysis and the surgical attempt to move the vocal fold into a more medial or lateral position. Indeed, the finding that the CAJ does not

undergo ankylosis after long-term neural deficits of the larynx has an impact on the efficacy of cord medialization procedures such as thyroplasty and others, as mentioned by Gacek and Gacek.²⁰

We conclude that long-standing RLNP does not result in ankylosis of the CAJ, but that the articular cartilage, the joint capsule, and the surrounding musculature of the CAJ undergo major structural changes with muscle atrophy, joint capsule shrinkage, and degenerative alterations of the articular cartilage comparable to those of osteoarthritis. These structural changes limit the recovery to normal joint function even if the RLNP resolves after several months. In the future, triggered electrical stimulation of the paralyzed muscles could be of value in decreasing the consequences of functional displacement of the vocal cords in cases of long-standing RLNP, thus creating a more favorable basis for secondary glottic surgery.

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EFFECTS OF VOICE THERAPY AS OBJECTIVELY EVALUATED BY DIGITIZED LARYNGEAL STROBOSCOPIC IMAGING

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Objective measurements derived from digitized laryngeal stroboscopic images were used to demonstrate changes in vocal fold vibration and in the size of benign lesions after 3 months of voice therapy. Forty chronically dysphonic patients were studied. By means of a rigid stroboscope, pretreatment and posttreatment recordings were made of the vocal folds at rest and under stroboscopic light during phonation. From each recording, images of the positions at rest and during vibration at maximal opening and at maximal closure were digitized. The surface areas of any lesions and of the glottal gap were independently measured in the digitized images by 2 experienced laryngologists. Referential distances were determined in order to compensate for discrepancies in magnification in the various recordings. After 3 months of voice therapy, significant improvement in lesion size and degree of maximal closure during vibration could be demonstrated in about 50% of the patients. The degree of maximal opening did not prove to be a significant parameter.

KEY WORDS — clinical practice, digital processing, quantification, treatment outcome, videostroboscopy, voice therapy.

INTRODUCTION

The expectation that the therapeutic effects of a treatment should be underpinned by scientific research is increasingly prevalent. A precondition for such research is the availability of relevant outcome variables. In the case of voice therapy, different sets of treatment outcome parameters provide us with diverse kinds of information concerning treatment effects. Common approaches in research on voice dysfunction are the perceptual evaluation of voice quality and acoustic analysis. Although perceptual measurements of breathiness and roughness have gained widespread acceptance as standard parameters in voice research, they remain essentially subjective in nature.

Laryngostroboscopy, however, enables the laryngologist to focus directly on the source of sound production — the vocal folds — and thus facilitates objective measurement. Videostroboscopy can be used as a tool for visual-perceptual evaluation of the glottal waveform and its abnormalities. For instance, a reduction in the dimensions of the glottal gap when the vocal folds are maximally closed may be observed as a result of voice therapy, as well as of laryngoplastic phonosurgery (see, among others, D'Antonio et al¹). Several protocols for evaluating data obtained with videostroboscopy have been developed (eg, Hirano and Bless,² Dejonckere et al,³ and Poburka⁴). Recent advances in the technique of digital processing of laryngeal images have enabled the develop-

ment of methods for deriving objective measurements from such endoscopic examinations (eg, Colton et al,⁵ Sercarz et al,⁶ Johnson et al,⁷ Omori et al,^{8,9} Gonçalves and Leonard,¹⁰ Hanson et al,¹¹ Jeannon et al,¹² and Björck and Hertegård¹³). Quantitative measurements derived from videolaryngostroboscopic images of vocal fold vibration in normal speakers of both sexes were published by Woo,¹⁴ and his reference data can be used to identify dysphonic patients as opposed to normal subjects. Hitherto, a handful of studies have addressed the evaluation of the effects of voice therapy by videolaryngostroboscopy (Kotby et al,¹⁵ Verdolini-Marston et al,¹⁶ Omori et al,⁸ and Bassiouny¹⁷). Omori et al⁸ have adopted new methods to measure dimensions on digitized laryngeal stroboscopic images.

The purpose of this study was to examine whether objective measurements derived from digitized laryngeal stroboscopic images can be used to detect significant changes in closure during vocal fold vibration and/or changes in the dimensions of benign lesions that may have occurred after 3 months of voice therapy. A majority of these benign vocal fold lesions are supposed to be related to voice abuse. The present study is part of a research project on the overall effects of logopedic voice therapy for patients as found in daily clinical practice. As a consequence, this study includes patients with a variety of indications for voice therapy and various approaches in voice ther-

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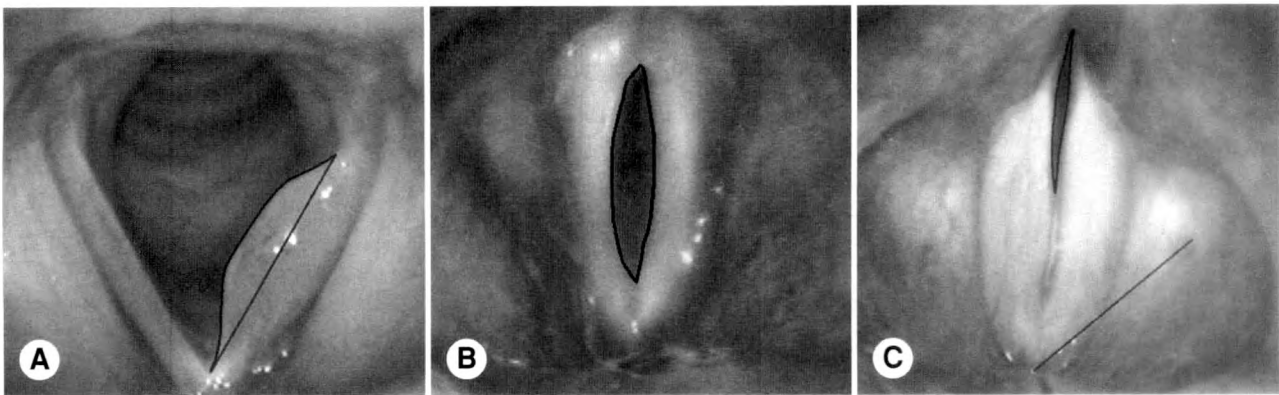


Fig 1. Measurements. **A)** Of surface area of lesion. **B)** Of surface area of maximal opening of vocal folds during phonation. **C)** Of surface area of maximal closing of vocal folds during phonation and corresponding referential distance (distance between telangiectasia and ventral commissure).

apy. This is in contrast to most studies in the literature, which concentrate on a specific patient population or therapy. As interaction between therapy and diagnosis is to be expected, the voice therapies were adapted to the diagnosis. The main goal was to investigate the efficacy of voice therapy in general. For ethical reasons, it was not possible in our experimental design to use a control group who did not receive any voice therapy. Therefore, only chronically dysphonic patients were selected to participate in our study. Because of the chronic nature of the dysphonic disorders, any change found on digitized laryngeal imaging could be considered to be the result of voice therapy.

METHODS

Subjects. The study reported in this paper is part of a larger study on the effects of voice therapy that uses a multidimensional assessment approach. Patients participating in this study were selected according to the following criteria: all patients had to have chronic dysphonia, the onset of the dysphonic problems had to have occurred at least 4 months before visiting an otorhinolaryngological specialist or a phoniatrician at the Phoniatric Department of the University Hospital Utrecht, and the exact medical diagnosis had to have been demonstrated by comprehensive phoniatric investigation. Those under 18 years of age were excluded from participating in this study because of possible problems of voice maturation or mutation. Furthermore, coexisting speech or language disorders, as well as malignant or premalignant lesions, hormonal voice disorders, laryngeal papillomas, gastroesophageal reflux, substitution of voice after laryngectomy, spasmodic dysphonia, and psychogenic dysphonia, were also exclusion criteria. The aim was to avoid, as far as possible, parameters other than voice therapy that would affect the outcome. If, according to the phoniatrician, voice therapy was indicated, patients were referred to speech

therapists in their residential area. The indications included trial therapy and presurgical voice therapy, whereby patients received voice therapy adjusted to their specific phoniatric diagnosis for at least 3 months, with a frequency of 30 minutes twice a week or 60 minutes once a week. Seventy-one subjects who satisfied the inclusion criteria without triggering the exclusion criteria completed 3 months of voice therapy.

A videostroboscopic recording was made before and after these 3 months, and of the 71 patients, both pretherapy and posttherapy stroboscopic recordings were suitable for further computerized analysis in 40 cases. Of this group, 36 subjects presented with pathological vocal fold closure, and 25 subjects with a benign vocal fold lesion. With 3 exceptions, all of the patients with lesions were categorized by a phoniatrician as also having glottal insufficiency. The main reason for not including a patient's data in the analysis was the inadequate quality of the video images concerned. The poor quality was caused by low light levels; involved images that were out of focus; or resulted from incomplete glottic images due to an overhanging epiglottis, prominent false vocal cords, or collapsed arytenoid cartilages (Jeannon et al¹²). Furthermore, because of the variable distance between the laryngoscope and the vocal folds, surface area measurements (in pixels) taken from vocal fold images require calibration to allow for variations in the magnitude of magnification. In order to place stroboscopic pretreatment and posttreatment recordings on the same scale, a referential distance was identified and measured (Fig 1).

The group of 40 patients consisted of 27 women and 13 men, with an age range of 18 to 76 years. The average age of the female subjects was 38 years, and that of the men was 52 years. The subjects represented a sample that reflects the diversity of laryngeal disor-

DISTRIBUTION OF PATIENTS BY DIAGNOSTIC CATEGORIES

<i>Phoniatric Diagnosis</i>	<i>N</i>
Muscle tension dysphonia	5
Submucosal swelling	7
Vocal fold edema (Reinke's edema)	2
Vocal fold nodules	7
Vocal fold polyp	3
Unilateral vocal fold paralysis	4
Other: slight vocal fold abnormalities*	9
Other: severe vocal fold abnormalities†	3

*Including chronic laryngitis, slight sulcus, contact ulcer, small (pseudo)cyst, etc.

†Including radiotherapy, scar, etc.

ders in chronic dysphonia as found in the clinical setting (see Table) and were used to evaluate the overall effects of voice therapy in such clinical practice. The chronic character of the dysphonia was confirmed by acoustic measurements on speech samples collected before therapy onset (baseline period) on 3 different days within 6 weeks. No significant systematic differences between these baseline measurements could be demonstrated.

Procedure. A rigid endoscope was used to achieve an image quality superior to that realized by flexible techniques of endoscopy. If necessary, a topical anesthetic (lidocaine hydrochloride 4%) was applied. Recordings on video tape (U-matic videocassette recorder Sony VO-5630) were made of vocal fold vibration during repeated stable phonation of a sustained vowel /a:/ or /i:/ at comfortable pitch and loudness with normal as well as stroboscopic light (Stroboscope RLS 9100, Kay Elemetrics Corp, Pine Brook, New Jersey). In addition, images of the vocal folds in the resting position were recorded.

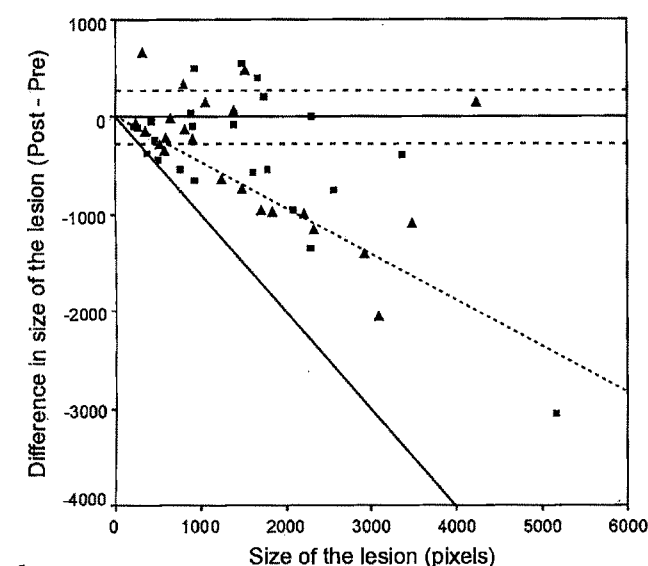
A personal computer (internal memory, 128 Mb) combined with a PCI videoadapter (ATI Technologies Inc, Thornhill, Canada; RAM memory, 8 Mb) served as a workstation. Selections of the video recordings were digitized and stored with a frame grabber (FastCap version 2.2.0 by FastMultimedia AG, Munich, Germany). By means of the software program VideoCapture (version 5.02 by Ulead Systems, Inc, Taipei, Taiwan), video frames were selected for further analysis. From each pretreatment and posttreatment recording, 3 images were captured. One image had been made during the resting position of the vocal folds to provide a clear image of any lesion present, and the other 2 images were taken during vibration of the vocal folds under stroboscopic light. The second image was chosen at the moment of the maximal opening of the vocal cords, and the third at the moment of maximal closure. The moment of maximal opening during vibration was considered to be a

global measure for the elasticity of the vocal folds, whereas the moment of maximal closure could reveal glottal insufficiency, which is presumed to be correlated to breathiness. Since quality was the criterion in selecting the images of maximal opening or closure from the whole recording, they need not have belonged to a single vibratory cycle. Three images were obtained from the pretreatment recordings, and 3 from the posttreatment recordings, so that 6 images per patient were available for quantitative measurement.

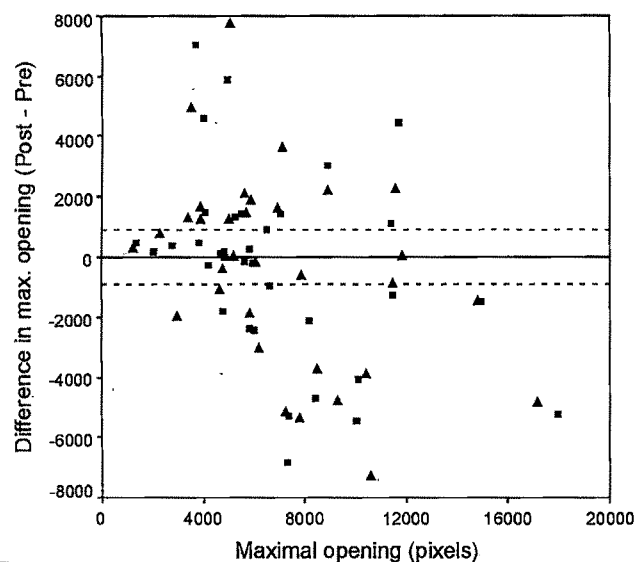
With the aid of the software program Scion Image (Scion Corporation, Frederick, Maryland), distances and surface areas were measured on the digitized images. Scion Image is an IBM-compatible version of a software program developed for Macintosh systems: NIH Image by W. Rasband (National Institutes of Health, Bethesda, Maryland). Measurements were made with a screen resolution of 1,024 horizontal pixels by 768 vertical pixels. Scion Image provides the ability to select the red, blue, and green component of an image separately and show it in shades of gray. In practice, green images shown in shades of gray appeared to produce the sharpest pictures of the vocal folds. In some cases, enhancement techniques were used to optimize the contrast and brightness of the images.

Contours of surfaces and distances of anatomic reference features were traced manually with a mouse. Areas within the traced contours were computed by the program. During the procedure, each patient's pretreatment and corresponding posttreatment images were shown on a monitor simultaneously without any indication as to which was which, and no sound track was provided. The area of the lesion was measured on the first image, the area of the maximal visible glottal gap during phonation was measured on the second image, and the area of the smallest gap was measured on the third image; all measurements were carried out independently by 2 experienced phoniatricians. A referential distance that was visible on the pretreatment and the posttreatment images was chosen and measured. Different kinds of referential distances were used, such as the distance between two blood vessels, the vocal fold length, and the width of an arytenoid cartilage or of the epiglottis. The error of intraobserver measurement was determined by independently performing the complete measurements twice, with an interval of at least 6 weeks. The intraobserver variance for the difference between the pretreatment and (corrected) posttreatment measurements was computed by means of an analysis of variance (Shrout and Fleiss¹⁸).

A correction was applied to the posttreatment values of the different parameters, to allow for discrep-

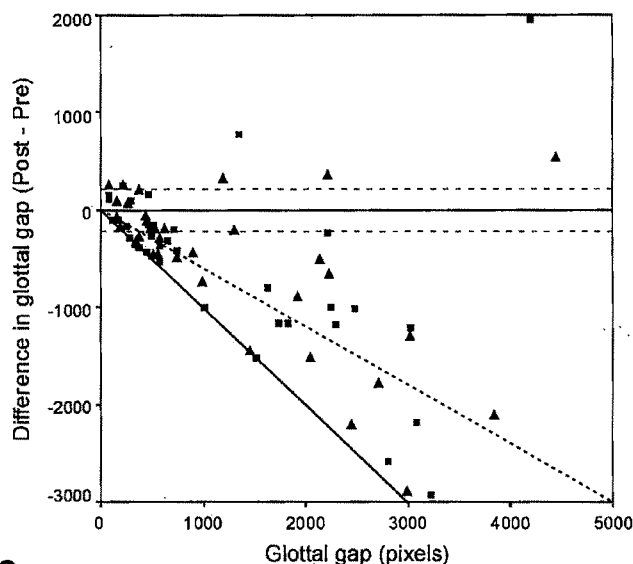


A



B

Fig 2. Data of rater 1 (squares) and rater 2 (triangles). **A)** Change in surface area of lesion after 3 months of voice therapy ($N = 25$) plotted against pretreatment surface area as assessed by 2 phoniatricians. Horizontal dotted lines define 95% confidence interval of intraobserver variability (± 270 pixels). Solid line represents position of values for patients in whom lesion had completely disappeared. Sloping dotted line is regression line through data below lower limit of confidence interval. **B)** Change in maximal opening of vocal folds during phonation after 3 months of voice therapy ($N = 35$) plotted against pretreatment maximal opening as assessed by 2 phoniatricians. Horizontal dotted lines define 95% confidence interval of intraobserver variability (± 888 pixels). **C)** Change in maximal closure of vocal folds during phonation after 3 months of voice therapy ($N = 36$) plotted against pretreatment glottal gap as assessed by 2 phoniatricians. Horizontal dotted lines define 95% confidence interval of intraobserver variability (± 212 pixels). Solid line represents position of values for patients in whom glottal insufficiency had completely disappeared. Sloping dotted line is regression line through data below lower limit of confidence interval.



C

ancies in magnification in pretreatment and posttreatment video recordings (see Appendix). Surface areas calculated from the posttreatment recordings were multiplied by a correction factor to correspond to the pretreatment recording data. This correction factor was the squared ratio of the referential distance as measured on the pretreatment recording to the same distance as measured on the posttreatment recording. Squared ratios were used because the videostroboscopic variables in this study are expressed as surface areas rather than linear distances. In order to determine the effect of voice therapy, we subtracted the pretreatment values from adjusted surface areas after therapy, so that quantified treatment effect data resulted.

RESULTS

A Wilcoxon matched-pairs signed-ranks test was used as an overall test for evaluating differences be-

tween pretreatment and posttreatment data. The results revealed that 1) the size of the laryngeal lesion and 2) the glottal insufficiency or area of the glottal gap in the maximal closure position of the vocal folds during vibration did show significant improvements ($p = .01$ and $p = .001$). The 25th, 50th, and 75th percentiles for change in the lesion were -52% , -36% , and -3% , and for change in maximal closure, these values were -88% , -46% , and -17% . The change in maximal opening position of the folds was not significant.

The treatment effects after 3 months of voice therapy in relation to the pretreatment data are specified for each patient in Fig 2. If no lesion or no air leakage in the closed position of the vibratory cycle was detected in the pretreatment examination, then these data were left out of the corresponding part of Fig 2 and analysis. The horizontal dotted lines in this Figure

represent the 95% confidence interval of the intraobserver variability of differences as assessed by means of an analysis of variance of the data of the repeated assessment. If no relevant differences were apparent between pretreatment and posttreatment videostroboscopic recordings, the corresponding data point was plotted between these lines. If, after 3 months of therapy, a lesion had completely disappeared, the data point was plotted on the sloping solid line in Fig 2A. In the case of maximal closure, this line represents a situation in which no air leakage occurs during closure of the vocal folds after therapy. Therefore, in all cases in which maximal possible improvement was achieved after therapy, the value concerned was plotted near the solid lines.

Figure 2 shows that in the case of the larger lesions, a decrease in the dimensions of the abnormality was generally achieved. Only a small number of patients had completely recovered after 3 months of therapy, and in some subjects the lesion did not change at all significantly as a result of therapy. Where small lesions were concerned, it was especially difficult to verify improvement in view of the margins of measurement error. In some 50% of the patients, a significant improvement was found. A regression analysis for the data below the lower limit of the 95% confidence interval of Fig 2A showed that the size of these lesions had been reduced on the average by about 46%. A complete recovery — that is, the state indicated by a posttreatment value within the confidence interval around the absence of a lesion — was found in 11% of the patients who had a lesion before therapy. Among the diagnostic categories, no clear differences in the pattern of recovery could be demonstrated in this study. A slight enlargement of the lesion was observed in 2 patients with vocal fold nodules and 2 patients with submucosal swelling.

The data on maximal opening of the vocal folds (Fig 2B) showed that an important group of subjects underwent an enlargement of this parameter, but a similar number of patients showed a reduction. This parameter did not systematically change as a result of 3 months of voice therapy.

Figure 2C shows that in the case of larger deviations that imply greater insufficiency of glottal closure, a decrease in the size of the abnormality was generally attained. As in those subjects who had lesions, in some 50% of the patients a significant improvement was demonstrated. Regression analysis for the data below the lower limit of the 95% confidence interval obtained in Fig 2C showed that the minimal glottal gap during vibration was reduced on the average by about 62%. A complete closure within the confidence interval was found in 20% of the pa-

tients with insufficient glottal closure before therapy. The diverse diagnostic categories showed similar patterns of recovery, except in the cases of 4 patients with vocal fold paralysis for whom the results of treatment were inconsistent. Deterioration of the maximal closing position of the vocal folds appeared unambiguously in 2 subjects with a diagnosis of unilateral vocal fold paralysis.

DISCUSSION

The following conclusions can be drawn from the results of this study. In the first place, objective measurements derived from digitized laryngeal stroboscopic images can be used to investigate the effects of voice therapy. It is evidently possible to use such measurements to evaluate whether voice therapy has positive effects on the functioning of the vocal folds. Measurements taken from laryngeal stroboscopic recordings before and after 3 months of voice therapy can provide valuable information concerning therapeutic effects and the usefulness of possible continuation of voice therapy.

Second, the valuable parameters on stroboscopic imaging appeared to be the size of a laryngeal lesion and the glottal insufficiency or area of the glottal gap during maximal closure of the vocal folds during vibration. No useful information was yielded by measuring the maximal opening position of the vocal folds. Third, it was demonstrated that in this group of chronically dysphonic patients with diverse disorders, a significant improvement after 3 months of voice therapy was realized in about 50% of the patients.

In this study, all videostroboscopic recordings made of vocal fold vibration during stable phonation were at comfortable pitch and loudness. Fundamental frequency and loudness are factors that influence vocal fold vibration. In normal phonation, an increase in sound intensity is positively correlated with glottal closure, although in female subjects, an increase in fundamental frequency with no transition among registers can be related to a decrease in closure (Sulter et al¹⁹). However, other authors did not find the degree of incomplete closure to be affected by changes in pitch (Södersten and Lindestad²⁰). The measurement data pertaining to treatment effect in this study may have been contaminated by a slight difference in pitch and loudness in the pretreatment recording as opposed to the posttreatment recording. Ideally, both parameters should be controlled, as would be feasible in a research laboratory with healthy subjects. However, this control would be difficult to achieve in a clinical setting in which the vocal disorders of patients are being assessed. Since, in this

study, pitch and loudness may not have been entirely consistent in the two sets of recordings, the results may show greater variability than they would have if these two parameters had been uniform on the pre-treatment and posttreatment recordings.

The degree of incomplete glottal closure is significantly higher in the case of normally speaking women than in men. Incomplete glottal closure of the posterior parts of the glottis during phonation should especially be regarded as normal in women, whereas complete closure appears to be the most common pattern in men (Södersten and Lindestad²⁰). Sulter and Albers²¹ showed that in women, a closure of at least 90% should be attained, so that a small glottal gap in female subjects should be considered as normal. In our study on pathological voice production, no significant differences could be found between treatment effects on maximal closure of the vocal

folds of female as opposed to male subjects. Although some female subjects may erroneously be considered to have had or still have an abnormal closure of the vocal folds, the observed effects will very likely be within the statistical margin of measurement error.

CONCLUSION

The results of this study show that objective measurements in digitized laryngeal stroboscopic images can be used to investigate the effects of voice therapy on the functioning of the vocal folds. In about 50% of the dysphonic patients in this study, a significant improvement after 3 months of voice therapy could be demonstrated. The size of the laryngeal lesion and the glottal insufficiency or area of the glottal gap in the maximal closure position of the vocal folds during vibration proved to be valuable parameters. In contrast, measuring the maximal opening position of the vocal folds failed to yield useful data.

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APPENDIX: IMAGE CALIBRATION FOR MAGNIFICATION DIFFERENCES OF PRETREATMENT AND POSTTREATMENT VIDEO RECORDINGS

In this study, the following correction procedure is used.

If r_1 = referential distance on pretreatment image

and

r_2 = referential distance on posttreatment image

then the correction factor (c) for discrepancies in magnification in pretreatment and posttreatment video recording is defined as:

$$c = (r_1/r_2)^2$$

The posttreatment surface area is multiplied by the correction factor to correspond to pretreatment data.

If a_1 = surface area on pretreatment image

and

a_2 = surface area on posttreatment image

then the adjusted surface area (a_2') is

$$a_2' = c \cdot a_2$$

The pretreatment data are subtracted from the adjusted surface area in order to determine the effect of voice therapy. The quantified treatment effect value (e) is

$$e = a_2' - a_1$$

HIGH-DOSE GLUCOCORTICOIDS INHIBIT PROLIFERATION OF RAT OLFACTORY EPITHELIUM

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Glucocorticoids (GCs) are commonly prescribed for treatment of olfactory dysfunction. However, the effects of GCs on olfactory epithelium are not well known. We investigated the effects of high-dose GCs on proliferating cells of olfactory epithelium. Five adult male rats (300 g) received a single daily subcutaneous dose of vehicle containing 0.3 mg dexamethasone (DEX) for 9 days (DEX+ group), and a control group received vehicle alone (DEX- group). We compared sections from the two groups for numbers of Ki67-positive cells. The mean number of Ki67-positive cells per 500 olfactory epithelial cells was 9.6 for the DEX+ group and 58 for the DEX- group (significant difference). We conclude that high-dose GC suppressed proliferation of olfactory epithelium. We suggest that high-dose GC suppresses cytokines and growth factors, resulting in secondary suppression of proliferating ability.

KEY WORDS — glucocorticoids, olfactory epithelium, proliferation.

INTRODUCTION

Glucocorticoids (GCs) are commonly used for the treatment of olfactory dysfunction.¹ However, the effects of GCs on the olfactory epithelium are not well known. The olfactory epithelium is unique in its ability to give rise to new sensory neurons throughout life, and neurogenesis is greatly enhanced after injury to the epithelium, allowing for rapid reconstitution of the damaged epithelium. Thus, proliferation of olfactory cells occurs continuously, even in adult animals.² In this experiment, we investigated the effects of high-dose GCs on proliferating cells in the rat olfactory epithelium.

MATERIALS AND METHODS

Five adult male rats (300 g) received a single daily subcutaneous dose of vehicle containing 0.3 mg dexamethasone (DEX) for 9 days (DEX+ group). We examined 5 adult male rats (300 g) that received only vehicle (DEX- group). Each rat was then decapitated to obtain specimens, and the specimens were decalcified in 10% ethylenediaminetetraacetic acid. The fixed specimens were embedded in paraffin and cut into 4- μ m-thick sections. After microwave treatment, the sections were incubated for 24 hours at 4°C with anti-Ki67 polyclonal IgG (Novocastra Laboratories, Newcastle, England) as the primary antibody. Immunohistochemical staining was performed with the LSAB kit (Dako Japan, Kyoto, Japan). Immunoreactive antibody-antigen complexes were visualized with 3,3-diaminobenzidine tetrahydrochloride.

After staining, we compared the two groups for numbers of Ki67-positive (Ki67+) cells in the sections.

RESULTS

In the olfactory epithelium of both groups, Ki67+ cells were identified mainly in the basal layer. The number of Ki67+ cells was smaller in the DEX+ group than in the DEX- group (Fig 1).

Under high-power magnification, the number of Ki67+ cells was smaller in the DEX+ group than in the DEX- group, both in the basal layer and in the surface layer of the olfactory epithelium (Fig 2).

The mean number of Ki67+ cells per 500 olfac-

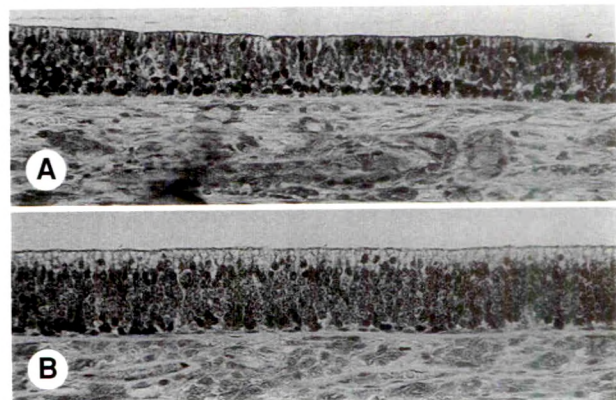


Fig 1. Olfactory epithelium of rats (diaminobenzidine and hematoxylin, original $\times 100$). **A)** Dexamethasone-negative (DEX-) group. **B)** Dexamethasone-positive (DEX+) group. Dark cells are Ki67-positive (Ki67+) cells.

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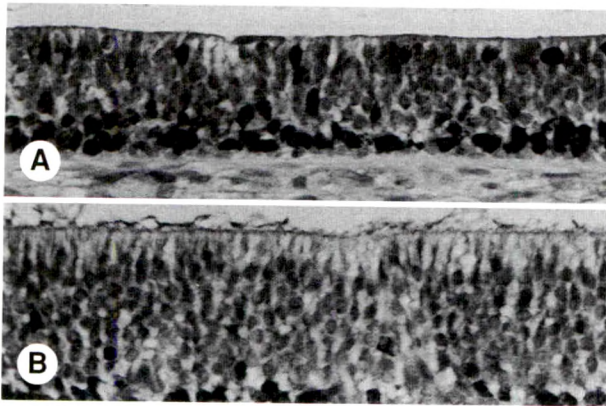


Fig 2. Olfactory epithelium of rats under high-power magnification (diaminobenzidine and hematoxylin, original $\times 400$). A) DEX- group. B) DEX+ group. Dark cells are Ki67+ cells.

tory epithelial cells was 9.6 in the DEX+ group and 58 in the DEX- group (Fig 3). This difference between the two groups was significant by the Aspin-Welch method.

DISCUSSION

Anti-Ki67 antibodies are used widely to show proliferating cells in all phases of the cell cycle (G1-, S-, G2-, and M-phase), but are absent from resting (G0-phase) cells.³ Thus, it is appropriate to identify proliferating cells in the olfactory epithelium with anti-Ki67 antibody.^{4,5} On the basis of our results, we suggest that high-dose GCs suppress proliferation of olfactory epithelium.

We consider the Ki67+ cells in the basal layer to be precursors of olfactory cells and the Ki67+ cells in the surface layer to be precursors of sustentacular cells. The number of Ki67+ cells decreased in the DEX+ group, both in the basal layer and in the surface layer of the olfactory epithelium. High-dose GCs suppressed the proliferation of both olfactory cells and sustentacular cells.

It is not clear why high-dose GCs suppressed proliferation of olfactory epithelium. Robinson et al⁶ found that GC receptor-like immunoreactivity associated with the olfactory epithelium was greatest at the apical surface, a position corresponding to the dendrites, knobs, and cilia of olfactory receptor neurons, as well as the supranuclear region of sustentacular cells. Weaker GC receptor-like immunoreactivity was associated with the region of cell bodies of the olfactory receptor cells. Within the lamina propria, the acinar cells of the Bowman's glands and the olfactory nerve bundle were intensely immunore-

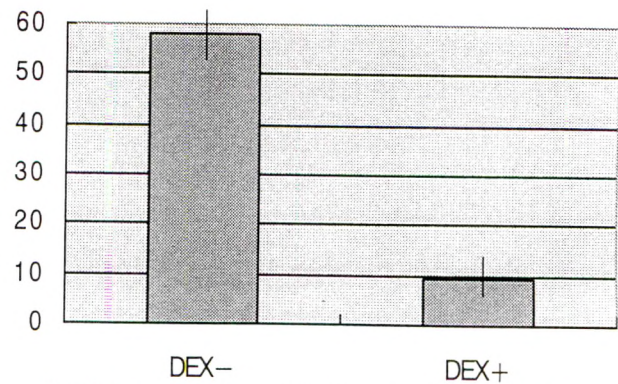


Fig 3. Mean numbers of Ki67+ cells in 500 olfactory epithelium cells: 9.6 in DEX+ group and 58 in DEX- group.

active. In their report, the basal layer of olfactory epithelium did not contain many GC receptors. Therefore, GCs may not suppress proliferation in the basal layer of the olfactory epithelium.

Glucocorticoids inhibit keratinocyte growth factor production in primary dermal fibroblasts.⁷ They may inhibit other growth factors. If GCs suppress cytokines and growth factors in the olfactory epithelium, this may explain why GCs suppress proliferation of olfactory epithelium. Because of olfactory epithelial exposure to the outside, olfactory epithelium consistently shows mild inflammation. Neurogenesis and proliferation of the olfactory epithelium may require mild inflammation. Therefore, if high-dose GCs suppress the inflammation, the ability to proliferate may decrease.

From the clinical point of view, GCs may help people who have lost their sense of smell. Why do GCs help patients with decreased olfactory ability? Most cases of anosmia and hyposmia are caused by inflammationlike chronic sinusitis and allergic rhinitis. We think that GCs decrease inflammation of the olfactory epithelium and increase the sense of smell. In this experiment, the dosage of GCs was much higher than the therapeutic dosage. Therefore, we need to investigate systemic administration of low-dose GCs.

CONCLUSION

We investigated the effects of high-dose GCs on proliferating cells in the rat olfactory epithelium. The number of Ki67+ cells in the rat olfactory epithelium was smaller in the DEX+ group than in the DEX- group. Although GCs are commonly prescribed for treatment of olfactory function, high-dose GCs can suppress normal proliferation of olfactory epithelium.

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EFFECT OF ACOUSTIC TUMOR EXTENSION INTO THE INTERNAL AUDITORY CANAL ON DISTORTION-PRODUCT OTOACOUSTIC EMISSIONS

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We studied the effects on distortion-product otoacoustic emissions (DPOAEs) of internal auditory canal (IAC) extension of acoustic neuromas (ANs) with the hypothesis that cochlear patterns of DPOAEs would be more commonly observed when the IAC was completely filled with tumor because of direct tumor involvement of either the inner ear or its blood supply. In a retrospective analysis of 86 patients with surgically proven ANs, DPOAEs were classified as having cochlear or noncochlear patterns on the basis of comparisons with the behavioral pure tone thresholds. The results of behavioral audiometry and DPOAEs were compared with the extension of the tumor into the IAC, which was categorized as full or partial. Of the 86 patients, 58 had tumors with full IAC extension, and 28 had tumors with partial IAC involvement. Cochlear patterns of DPOAEs were found in 55.2% of the tumors in the full IAC group and in 71.4% of those in the partial IAC group (not statistically different). It was concluded that the extent of IAC involvement by ANs was not significantly related to the negative effects of the tumor on cochlear function as represented by DPOAEs.

KEY WORDS — acoustic neuroma, internal auditory canal, otoacoustic emissions.

INTRODUCTION

Evoked otoacoustic emissions (OAEs) are low-level acoustic signals of cochlear origin that can be recorded in the external auditory canal with a sensitive microphone after the delivery of an appropriate acoustic stimulus.¹ They are thought to arise as a by-product of a biomechanical feedback loop in which the outer hair cells (OHCs) participate to enhance basilar membrane vibration. This active cochlear processing is thought to originate with the miniscule movements of the OHCs, which have been shown to exhibit micromechanical motility when stimulated electrically in vitro.² Measurement of OAEs provides a noninvasive indication of the integrity of this pre-neural biomechanical amplification process.³ Thus, in the case of sensory hearing loss, when the OHCs are compromised, OAEs are reduced or absent.

It is generally accepted that acoustic neuromas (ANs) cause hearing losses resulting from dysfunction in either the peripheral (cochlea) or the central (neural) auditory structures or both. The mechanisms by which ANs damage the cochlea remain unclear, but it has been generally assumed that a major cause is reduction in cochlear blood flow. In previous investigations, the effect of an AN on hearing was con-

sidered to be “cochlear” when evoked OAEs were reduced in tandem with the behaviorally measured hearing thresholds.⁴ On the other hand, the effect of tumor was considered to be “noncochlear” when evoked OAEs were within the normal range or better than expected on the basis of the hearing loss.

Bonfils and Uziel⁵ analyzed transiently evoked otoacoustic emissions (TEOAEs) in patients with ANs and obtained TEOAEs in 9 of 28 ears with tumors. Of the 9 ears with intact TEOAEs, 5 had average hearing thresholds of greater than 25 dB hearing level (HL) in the ranges between 0.5 and 2 kHz. This amount of hearing loss in the presence of TEOAEs implied a “retrocochlear” (ie, noncochlear) site of lesion in the auditory pathway. However, the authors reported that the TEOAE detection thresholds were similar to those in other (nontumor) ears with cochlear losses and concluded that ANs usually produce a cochlear hearing loss.⁵ In contrast, Ohlms et al⁶ observed that patients with ANs exhibited only mildly reduced distortion-product otoacoustic emissions (DPOAEs) even in the presence of appreciable hearing losses. These findings were consistent with a retrocochlear basis for the hearing loss. Thus, these authors suggested that evoked OAEs could make a

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major contribution toward differentiating the sensory component from the neural component of hearing impairment.⁶

Based on past studies of DPOAEs in AN patients, the present study was undertaken to assess the relationship between the extent of the tumor in the internal auditory canal (IAC) and evoked DPOAE activity. In particular, the goal was to determine whether the extension of the AN into the IAC significantly increased the likelihood that an ear would be classified as having a cochlear pattern of DPOAEs.

MATERIALS AND METHODS

Data were collected from the records of patients who received a diagnosis of AN between January 1992 and November 1999. Pure tone averages (PTAs) and speech discrimination scores (SDSs) were obtained from reports of behavioral audiometry. The AN tumor size was measured as the largest diameter of the mass as seen on either the axial or coronal magnetic resonance imaging scans. The DPOAEs were obtained only when tympanometry indicated normal middle ear compliance (ie, type A). Patients with conductive hearing loss or abnormal immittance findings were excluded from the study because of the known adverse effects of middle ear dysfunction on DPOAEs. In addition, only patients who underwent surgical treatment for unilateral AN and had a positive pathological confirmation of schwannoma were included. Behavioral PTAs, SDSs, speech reception thresholds, and acoustic immittance were determined for all patients by conventional diagnostic audiological procedures. Behavioral pure tone thresholds were obtained at frequencies of 1, 2, 4, and 6 or 8 kHz. The final study group included 86 patients who met the inclusion criteria.

The DPOAE subtype of OAEs was used because previous work suggested that in the presence of mild to moderate hearing loss, DPOAEs tended to be more robust than click-evoked TEOAEs.^{7,8} Specifically, DPOAEs could be measured in ears exhibiting cochlear losses up to about 45 to 55 dB HL, as compared to a 30-dB HL limit for detecting click-based TEOAEs. In addition, DPOAEs can be elicited at higher test frequencies of up to 8.0 kHz.

Measurement of DPOAEs. Measurement of the DPOAEs by experienced practitioners took place in either a human OAE research laboratory or in a clinical audiology suite. Briefly, in the research laboratory, DPOAEs at 2f₁-f₂ were measured with a commercially available acoustic probe (ER-10 or ER-10B, Etymotic Research, Elk Grove, Illinois) incorporating several miniature microphones and sound tubes from 2 externalized ear speakers (ER, Etymotic

Research). Stimulus generation and response acquisition were controlled by customized microcomputer-based software operating an onboard digital signal processor (Macintosh IIfx, Apple Corp, Cupertino, California). The DPOAEs were obtained with equal level (L₁ = L₂) primary f₁ and f₂ tones (f₂/f₁ = 1.21) at 75 dB sound pressure level. The geometric mean (GM) frequencies of the primaries [$GM = (f_1 \times f_2)^{0.5}$] were distributed in 0.1-octave steps, from 0.8 to 8.0 kHz. As part of the data collection procedure, the amplitudes for 31 GM frequencies were automatically entered into a commercial spreadsheet (Microsoft Excel) along with the levels of the related noise floors (ie, the average amplitude of 5 frequency bins on either side of the DPOAE frequency).

In the audiology suite, DPOAEs were measured with the commercially available ILO 92 System (Otodynamics Ltd, Hatfield, England), which generated DPOAE amplitude measures at GM frequencies distributed in one-third-octave steps, from 0.5 to 6.0 kHz, ie, at 3 points per octave. We obtained DP-grams using equilevel settings of L₁ = L₂ = 75 dB sound pressure level for f₁ and f₂ primary tones.

Cochlear Versus Noncochlear Classification. Detailed methods for classification of DPOAEs in ears with tumors have been described elsewhere.⁴ Briefly, subjective classifications were performed by a qualitative approach that related DPOAEs to the corresponding behavioral hearing thresholds. On the basis of our previous experience, the DPOAE results were categorized into cochlear and noncochlear categories by comparing the emission amplitude to the corresponding hearing level at the same test frequencies (ie, 1, 2, 4, and 6 or 8 kHz). For example, ears with behaviorally measured hearing loss (ie, hearing loss of >20 dB HL), and DPOAEs that were reduced accordingly, were assigned to the cochlear group. Thus, for ears with hearing loss categorized as cochlear, diminished DPOAE levels were associated with impaired hearing. In contrast, ears designated as having noncochlear hearing loss displayed DPOAE levels that were either within the normal range or better than expected on the basis of the accompanying behavioral hearing loss. To control for the general effect of associated sensorineural hearing loss on DPOAE levels (eg, hearing loss related to presbycusis and noise exposure), we also took into account the relationship between hearing threshold and DPOAEs in the nontumor ear before making a final assignment to either the cochlear category or the noncochlear category. Next, the tumor's IAC involvement and/or its lateral extension was determined according to intraoperative findings. For purposes of this study, the tumor's IAC extension was considered either full or partial, depending on whether the

TABLE 1. COMPARISON OF CHARACTERISTICS OF PATIENTS WITH FULL VERSUS PARTIAL EXTENSION OF ACOUSTIC NEUROMA INTO INTERNAL AUDITORY CANAL

Characteristic		Overall (n = 86)	Full Extension (n = 58)	Partial Extension (n = 28)	p
Gender (male)	No.	42	28	14	.881
	%	48.8	48.3	50.0	
Site (left ear)	No.	38	27	11	.525
	%	44.2	46.6	39.3	
Age (y)	Mean $\pm$ SD	54.9 $\pm$ 13.5	52.8 $\pm$ 13.8	56.3 $\pm$ 12.9	.255
	Range	14.0-78.0	14.0-72.0	27.0-78.0	
Tumor size (cm)	Mean $\pm$ SD	2.1 $\pm$ 1.1	2.1 $\pm$ 1.1	2.0 $\pm$ 1.1	.668
	Range	0.5-5.5	0.5-5.5	0.5-4.0	
Pure tone average (dB)	Mean $\pm$ SD	56.4 $\pm$ 29.5	60.5 $\pm$ 30.8	48.0 $\pm$ 25.1	.066
	Range	8.8-120.0	8.8-120.0	12.5-111.3	
Speech discrimination score (%)	Mean $\pm$ SD	44.9 $\pm$ 38.2	39.9 $\pm$ 36.6	55.3 $\pm$ 40.1	.077
	Range	0.0-100.0	0.0-100.0	0.0-100.0	

tumor extended into the lateral third of the IAC. Once the classification was complete, the above cochlear and noncochlear groups were compared according to whether the tumor extension was partial or full.

Statistical Methods. Statistical analyses were performed with Statistix (Analytical Software, Tallahassee, Florida). Descriptive statistics were obtained for all variables, and if necessary, appropriate transformations were used to normalize the data before parametric statistical testing. Comparisons with respect to age, tumor size, PTAs, and SDSs of patients with full versus those with partial IAC tumor extension were performed with independent sample *t*-tests or, in the case of variables with extreme non-normality such as the SDS, the Mann-Whitney test. Associations between extension categories and discrete variables such as DPOAE type, gender, and site were assessed by χ^2 tests. Separate univariate logistic regression analyses were used to assess the effect of each potential predictor of DPOAE category (cochlear versus noncochlear). Finally, multiple logistic regression analyses were performed to assess the independent effect of each potential predictor of DPOAE category after adjusting for the effect of the remaining independent variables included in the model.

RESULTS

Characteristics of the patients (mean $\pm$ SD, range, and percentages) are presented in Table 1 for the en-

tire group (n = 86) and by tumor extension (full versus partial). Patients with full IAC extension were younger, had slightly larger average tumor size, and had higher PTAs and lower SDSs. None of the differences was statistically significant, but differences in PTA and SDS approached significance (p = .066 and p = .077, respectively).

Table 2 shows the cross classification of the study patients by the extension of the AN into the IAC (full versus partial) and the DPOAE pattern (cochlear versus noncochlear). Overall, the DPOAEs were classified as cochlear in 52 cases (60.5%). Among patients with full IAC tumor extension, 55.2% (32/58) had cochlear-type patterns of DPOAEs, whereas in patients with partial IAC tumor extension, the corresponding proportion was 71.4% (20/28). However, the difference between these proportions was not statistically significant (p = .149).

Separate bivariate logistic regression analyses, with type of DPOAEs as the dependent variable, were performed (details not shown). None of the results was statistically significant, but the odds of cochlear-type DPOAEs were higher for males, right ears, larger tumor sizes, higher PTA levels, and partial extension (odds ratio, 2.04; 95% confidence interval, 0.77-5.26). These results for the effect of the tumor extension on the DPOAE pattern remained virtually unchanged when we used several multiple logistic regression models.

DISCUSSION

The primary finding of this study was that the patterns of DPOAEs (cochlear and noncochlear) were not affected by the degree of IAC involvement by the ANs. Patients with tumors with full IAC extension had a lower proportion of cochlear DPOAEs than did those with partial IAC extension. When the tumor filled the IAC (ie, full IAC extension), a little more than half of the patients (55.2%) had cochlear-

TABLE 2. CROSS CLASSIFICATION OF PATIENTS (N = 86) BY EXTENSION OF ACOUSTIC NEUROMA INTO INTERNAL AUDITORY CANAL AND PATTERN OF DISTORTION-PRODUCT OTOACOUSTIC EMISSIONS

Pattern	Full Extension		Partial Extension		Total Extension		p
	No.	%	No.	%	No.	%	
Cochlear	32	55.2	20	71.4	52	60.5	.149
Noncochlear	26	44.8	8	28.6	34	39.5	
Total	58	100.0	28	100.0	86	100.0	

type patterns of DPOAEs. Unexpectedly, when the tumor extension in the IAC was partial, a majority of patients (71.4%) had a cochlear pattern of DPOAEs. This difference was not statistically significant. Although, in cases of full IAC extension, there was a tendency toward increased PTA thresholds (60.5 dB versus 48 dB) and lower SDSs (39.9% versus 55.3%), the differences were only marginally statistically significant. Furthermore, tumor size was not associated with the DPOAE pattern, although hearing loss was marginally correlated to tumor size, as has been found in other series.^{4,9,10} It has been generally accepted that ANs cause hearing losses that result from dysfunction in both the peripheral (cochlea) and central (neural) auditory structures. The central effects have been presumed to be due to direct physical pressure on, and atrophy of, the eighth cranial nerve and the cochlear nucleus as seen in some pathological specimens.^{11,12} The sensory component has been theorized to arise from any one or a combination of the following pathophysiological mechanisms: vascular compromise, hydrops, degradation of inner ear fluids from tumor toxicity, deafferentation, and loss of efferent tuning.^{11,13,14} Of these possible explanations for the effects of ANs on sensory hearing loss, vascular compromise has been the most prominent notion.¹⁵

It was hypothesized for this study that when the fundus of the IAC was involved by, or when the entire canal was filled with, AN tumor, cochlear patterns would be more frequently observed. This hypothesis was based on the assumption that the tumors that caused reduction in cochlear function would

need physical proximity to either the inner ear or the internal auditory artery, or both. The fundus and lateral portion of the IAC were free of disease in all those tumors categorized as having a partial IAC extension. However, the lack of significant differences in any of the patient or tumor involvement characteristics between the cochlear and noncochlear groups suggested that the ANs exerted their negative effects on the cochlear component of hearing and OAEs in a manner that was independent of the extent of IAC involvement. In fact, the results of this study suggested a trend for noncochlear patterns of DPOAEs to occur more frequently than cochlear patterns when the IAC was full of tumor (71.4% versus 55.2%), suggesting that pressure on or invasion into the cochlear nerve by the tumor was more commonly the pathophysiological mechanism of hearing loss in these cases. These results suggested that direct toxicity via proximity or microinvasion into the inner ear is less likely to be the mechanism of cochlear dysfunction, or at least, that close physical proximity of tumor to the vestibule or inner ear is not necessary for the AN to negatively affect cochlear function. This idea favors a retrograde transport or some remote mechanism whereby the inner ear can be affected by the tumor without physical contact.

CONCLUSION

The DPOAEs of patients with ANs demonstrate either cochlear or noncochlear patterns of acoustic emissions. In this study, these DPOAE patterns were not significantly associated with the degree of AN tumor involvement in the IAC.

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LARYNGEAL TUBERCULOSIS: A REPORT OF 15 CASES

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Laryngeal tuberculosis is usually a complication of pulmonary tuberculosis. Recent studies have described a change in the clinical features of laryngeal tuberculosis. We present 15 cases of laryngeal tuberculosis treated at the Osaka Prefectural Habikino Hospital between 1993 and 2000. The results showed a mean age of 51 years, a male predominance by 2.75 to 1, and a 20% incidence ($n = 3$) of negative chest radiographic findings. The prominent presenting symptom was hoarseness (73.3%), and systemic symptoms were relatively rare. Seven patients showed ulcerative lesions, 5 showed granulomatous lesions, and the remaining 3 showed nonspecific inflammatory lesions in the larynx. Laryngeal lesions did not show any predilection for specific laryngeal sites in our series. In contrast to earlier studies, our study shows variations in clinical features of laryngeal tuberculosis. Physicians should consider tuberculosis in the differential diagnosis of laryngeal disease.

KEY WORDS — hoarseness, laryngeal tuberculosis, larynx.

INTRODUCTION

In the preantibiotic era, laryngeal tuberculosis was considered the most common disease of the larynx, affecting 37% of patients with pulmonary tuberculosis.¹ Laryngeal tuberculosis has become quite rare with the advent of antituberculosis chemotherapy. However, since 1990, the topic has gained new interest, because of the newly rising incidence and changing behavior of the disease.^{2,3} The emergence of acquired immunodeficiency syndrome and other immunosuppressive diseases or treatments will further increase the incidence and spectrum of tuberculosis.⁴ The aim of this study is to evaluate the changing trends in clinical manifestations of laryngeal tuberculosis.

MATERIAL AND METHODS

The records of all patients admitted to Osaka Prefectural Habikino Hospital between 1993 and 2000 with the diagnosis of laryngeal tuberculosis were reviewed, and 15 cases were identified. Criteria for diagnosis were histologic examination of biopsy specimens, positive smear tests and sputum cultures, and response to tuberculosis therapy. The patients' records were reviewed for the presenting symptoms, site and appearance of the lesion, chest X-rays, histologic examinations, smear tests, sputum cultures, poly-

merase chain reaction (PCR) tests, and purified protein derivative tests.

RESULTS

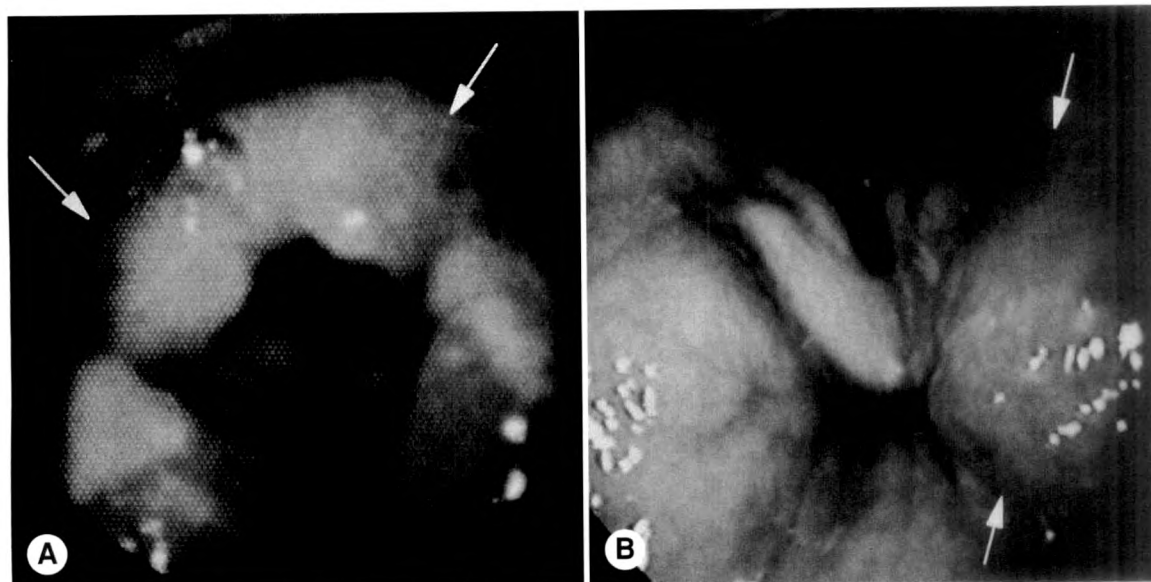
The patients ranged in age from 25 to 78 years, with an average of 51.1 ± 3.7 years (mean $\pm$ SE), and included 11 men and 4 women (male-to-female ratio, 2.75 to 1). The symptoms encountered, in order of frequency, included hoarseness (73.3%, $n = 11$), cough (66.7%, $n = 10$), odynophagia or pharyngodynia (60.0%, $n = 9$), weight loss (46.7%, $n = 7$), and dysphagia (26.7%, $n = 4$).

The various fiberoptic findings (see Figure) were categorized into 3 different appearances: ulcerative lesions ($n = 7$), granulomatous lesions ($n = 5$), and nonspecific inflammatory lesions ($n = 3$; Table 1). All ulcerative lesions manifested as multiple lesions. However, most granulomatous lesions were single lesions (Table 1; χ^2 test, $p < .05$). The ulcerative lesions did not show a predilection for any specific laryngeal site, whereas granulomatous lesions commonly involved the epiglottis, true and/or false vocal cords, and ventricle (Table 2). In 4 cases (3 with ulcerative lesions and 1 with granulomatous lesions), carcinoma of the larynx was the presumed diagnosis on admission.

The results of the investigations are presented in

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Laryngeal tuberculosis. **A)** Ulcerative lesions (arrows) of true and false vocal cords, ventricles, and arytenoid cartilages in 46-year-old woman. **B)** Granulomatous lesions (arrows) of left-side false vocal cord in 69-year-old man.

Table 3. Twelve of the 15 cases showed radiographic evidence of active pulmonary tuberculosis. A biopsy specimen was obtained from 8 patients, and only 4 patients showed histologic features of tuberculosis. In 1 of the 4 histologically negative cases, 3 separate biopsies all demonstrated nonspecific results.

Most of the patients with ulcerative and granulomatous lesions showed active pulmonary disease, whereas most of the patients with nonspecific inflammatory lesions showed normal lung status (Table 4; χ^2 test, $p < .001$).

DISCUSSION

Laryngeal tuberculosis continues to occur despite extensive tuberculosis eradication campaigns. Since 1997, after declining in incidence for decades in Japan, tuberculosis has again shown a worrisome steady increase.⁵ Therefore, laryngeal tuberculosis is likely to be encountered by an increasing number of physicians. Most physicians, however, do not consider tuberculosis in the differential diagnosis of various laryngeal symptoms, and this oversight leads to misdiagnosis and inappropriate treatment.²

In the past, tuberculosis was usually encountered

TABLE 1. CATEGORIZED FINDINGS OF FIBEROPTIC LARYNGEAL EXAMINATION

Fiberoptic Findings	Single Lesions	Multiple Lesions	Total	
			No.	%
Ulcerative	0	7	7	46.7
Granulomatous	4	1	5	33.3
Nonspecific inflammatory	2	1	3	20.0
Total	6	9	15	

$\chi^2 = 8.89$, $p < .05$.

in men under 40 years of age. More recent reports have indicated that patients presenting with laryngeal tuberculosis tend to be in the fifth to sixth decades.⁶ The mean age of our population was 51.1 years. The predominance of males among patients has long been accepted, with most reports showing a male-to-female ratio ranging from 2:1 to 3:1.^{3,7} A ratio of 2.75 to 1 was observed in our series.

The clinical picture of laryngeal tuberculosis has changed. Previously, patients demonstrated advanced pulmonary disease with a productive cough and marked constitutional symptoms such as fever, weight loss, and night sweats.^{7,8} During the past 2 decades, the incidence of constitutional symptoms has been variable. The main presenting symptom in this study was hoarseness, in accordance with recent reports.⁶⁻⁸ In this study, systemic symptoms were relatively rare.

The laryngeal lesions showed no predilection for any laryngeal sites in our cases, as noted by other authors.^{6,8,9} Posterior laryngeal involvement (arytenoid) due to pooling of the infected sputum in a recumbent patient, although classically described,⁶ did not predominate. Some authors attribute anterior la-

TABLE 2. TUBERCULOUS LESION SITES INVOLVED IN LARYNX IN FIFTEEN CASES

	Epiglottitis	True Vocal Cord	False Vocal Cord or Ventricle	Arytenoid
Ulcerative	6	5	5	5
Granulomatous	2	3	2	1
Nonspecific inflammatory	0	2	1	1

TABLE 3. INVESTIGATIONS IN FIFTEEN CASES OF LARYNGEAL TUBERCULOSIS

	Chest X-ray	Histology	Smear Test	Culture	PCR	PPD
Positive	12	4	9	14	4	6
Negative	3	4	6	1	0	2
No record	0	7	0	0	11	7

PCR — polymerase chain reaction test; PPD — purified protein derivative test.

ryngeal involvement (epiglottic) to the predominance of lymphatic or hematogenic spread, rather than direct spread from advanced pulmonary lesions.^{3,8,10} This mechanism may account for the 20% incidence of negative chest radiographic findings seen in this study.

Recent studies have shown that in the majority of cases, the lesion is hypertrophic or even exophytic and is not easily distinguished from chronic laryngitis.^{2,3,10} In our cases, however, ulcerative lesions were predominant, with involvement of multiple sites. The appearance of laryngeal tuberculosis has been variable. Thaller et al⁷ suggested that it depends upon the location of the lesions. If the mucosa is adherent to the underlying structures, as in the true vocal cord area, ulceration is a common manifestation.

It has been reported that laryngeal tuberculosis occurs with a lesser degree of pulmonary involvement.² In our study, when the lesion appeared to be more nonspecific, there was less incidence of pulmonary involvement, and when the lesion appeared to be more ulcerative and granulomatous, there was greater incidence of active pulmonary involvement. This finding implies that the clinical changes of laryngeal tuberculosis are linked with lesser or minimal pulmo-

TABLE 4. STATUS OF LUNG ACCORDING TO FIBEROPTIC FINDINGS

Lung Status	Ulcerative	Granulomatous	Nonspecific Inflammatory	Total
Active disease	6	5	1	12
Normal	1	0	2	3
Total	7	5	3	15

$\chi^2 = 15.00$, $p < .001$.

nary alterations. Our results agree with the report by Shin et al.³

Positive histologic findings were noted in only 4 of 8 cases. In 1 case with an ulcerative lesion, 3 separate biopsies all demonstrated nonspecific results. Although a high rate of positivity was found on sputum culture and PCR testing, sputum culture generally requires several weeks, and PCR testing may give false-positive results.¹¹ Because each examination has advantages and disadvantages, physicians should combine and repeat examinations for definitive diagnosis of laryngeal tuberculosis.

The typical patient with laryngeal tuberculosis is also at high risk for laryngeal carcinoma. Moreover, laryngeal carcinoma is more common than laryngeal tuberculosis.⁹ The danger of misdiagnosis of laryngeal tuberculosis as laryngeal cancer is evident.² In our study, 4 patients were initially considered to have neoplastic lesions of the larynx. Physicians should consider tuberculosis in the differential diagnosis of laryngeal disease. Early diagnosis is important for prompt treatment and to minimize both possible complications in the patient and excessive exposure to hospital personnel.⁷

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IMMORTALIZATION OF RAT EUSTACHIAN TUBE EPITHELIAL CELLS BY ADENOVIRUS 12–SIMIAN VIRUS 40 HYBRID VIRUS

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The eustachian tube epithelial cells play an important role in the initial pathogenesis of otitis media. In order to study the role of the eustachian tube epithelial cells in the pathogenesis of otitis media, we have established a rat eustachian tube epithelial cell line. The cell line was derived by infecting primary cultures of eustachian tube epithelial cells with the adenovirus 12–simian virus 40 (Ad12-SV40) hybrid virus. The immortalized cells have retained the morphological characteristics of the parental cells and show positive staining with anti-cytokeratin antibodies (a marker for epithelial cells), but not with anti-vimentin antibodies (a fibroblast marker). The cells have been in continuous culture for more than 10 months and have undergone 38 passages. Western blotting and cell staining have confirmed the expression of the SV40 T antigen and p53. Chromosomal analysis indicates that the cell line is aneuploid and derived from male rat epithelial cells. Together, our results suggest that the cell line originated from eustachian tube epithelial cells from a male rat and was successfully immortalized by the Ad12-SV40 virus.

KEY WORDS — adenovirus 12–simian virus 40 hybrid virus, cell line, eustachian tube epithelial cell, immortalization, primary culture.

INTRODUCTION

It is believed that the normal mucosal functions of the eustachian tube provide the first line of defense against otitis media and that the epithelial cells of the eustachian tube play a very important role in maintaining the sterility of the middle ear. The tube is the portal through which pathogens of the upper respiratory tract can gain access to the middle ear cavity, and there is abundant epidemiological and clinical evidence supporting the idea that the dysfunction of the mucosal defense system of the eustachian tube, including the dysregulation of periciliary fluid, is an important predisposing factor in the pathogenesis of otitis media.¹ Under normal conditions, eustachian tube epithelial cells secrete antimicrobial molecules, as well as surfactants, and regulate the periciliary fluid that is necessary for the proper functioning of the mucociliary system of the eustachian tube.²⁻⁵

Although much stands to be gained from the understanding of the molecular basis of the changes that eustachian tube epithelial cells undergo during the pathogenesis of otitis media, our knowledge in this area remains limited. This has been, in part, due to the difficulty in obtaining sufficient amounts of eustachian tube tissue, thus rendering the task of es-

tablishing primary cultures of the epithelial cells very labor-intensive. Furthermore, once established, the limited life span of the primary cultures makes these cells suboptimal for studies that require long-term culture or those that require large numbers of cells. The availability of the immortalized eustachian tube epithelial cells will help to increase our understanding of the normal defense mechanisms of the eustachian tube that make the tube an effective barrier against pathogens. Such mechanisms include the expression of the myriad of protective molecules or changes in the interactions between tubal epithelial cells and pathogens. The cell line will also enable us to carry out in vitro studies such as those aimed at analyzing global gene or protein expression patterns in response to pathogens or other stimuli. The results obtained from such experiments could potentially lead to the identification of key molecules whose modulation or manipulation may open new avenues for the therapy of otitis media.

In the past several years, investigators have established primary cultures and immortalized lines of middle ear epithelial cells from a variety of species.⁶⁻⁹ There have been no reports, however, of primary cultures or immortalized lines derived from eustachian

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tube epithelial cells. In this study, we report the first successful establishment and characterization of a rat eustachian tube epithelial cell line (RETEC).

MATERIALS AND METHODS

Animals and Culture Media. A total of 25 healthy rats (Wistar-Kyoto; body weight, 200 to 300 g) without middle ear disease were supplied from Taconic (Germantown, New York) for this study. The culture medium used consisted of LHC-8 medium (Biofluids, Inc, Rockville, Maryland) supplemented with 2% fetal bovine serum (Gibco, Gaithersburg, Maryland), retinoic acid (3.3×10^{-6} mol/L; Sigma, St Louis, Missouri),¹⁰ penicillin G (100 µg/mL), streptomycin (100 µg/mL), and amphotericin B (0.25 µg/mL).

Primary Cultures. Under ketamine hydrochloride anesthesia, the rats were painlessly sacrificed by cervical dislocation and decapitated. The head of the rat was cut open along the sagittal midline under a dissection microscope. The inner side of the bulla and the eustachian tube were dissected out, and the tube with the tubotympanic orifice was cut off. The tube was cut open from the orifice, and the mucosal explants of the tube were harvested. The explants were immersed in normal saline solution with antibiotics (penicillin G 100 µg/mL, streptomycin 100 µg/mL, amphotericin B 0.25 µg/mL; Sigma) for 1 hour at 4°C in order to minimize the possibility of bacterial contamination. The explants were plated in 35-mm tissue culture dishes coated with collagen type I (Biocoat, Bedford, Massachusetts) and incubated with a small amount of culture medium at 37°C in 5% carbon dioxide. After 24 hours, 2 mL of the culture medium was added to each dish. When outgrowths were observed, the culture medium was changed twice per week for continuing growth. By the 10th day, all explants were removed. If fibroblasts were observed in the outgrowth, they were physically removed with a surgical scalpel under the microscope.

Infection With Ad12-SV40 Hybrid Virus. Once at 80% confluence, the cultures were infected with 0.2 mL of adenovirus 12-simian virus 40 (Ad12-SV40) hybrid virus (1:10 dilution) per dish and incubated for 72 hours. The culture medium was then removed, and 2 mL of fresh medium was added to each dish. These cells were allowed to grow to 100% confluence and were then subcultured. From passages 7 to 9, a cytopathic effect assay was used to detect viral infection.¹¹ Briefly, the supernatant of the virus-infected eustachian tube epithelial cells was inoculated into Vero cell cultures. At day 21, if the Vero cells died or contained sick cells, the culture was determined to be a virus producer.

Single-Cell Clones. This procedure was carried out for the virus-infected rat eustachian tube epithelial cells at passage 10 by use of the limiting dilution method. The virus-infected cells were trypsinized and then washed with phosphate-buffered saline solution (PBS; pH 7.2). The cells were then resuspended in PBS at a concentration of 3 cells per milliliter. Then, 0.3 mL of the cell suspension was added to each well of a microtiter plate (96 well). After 6 hours, the wells were observed under the microscope, and all single-cell wells were selected for continuous culture. The clone with the best growth characteristics was selected and designated as the RETEC.

Proliferation and Doubling Time. In order to determine the proliferative ability of the cells, we constructed a growth curve and calculated the doubling time (DT) for the RETEC line. Briefly, 1×10^5 cells were seeded per 60-mm Petri dish. With a hemocytometer, the cell number in 3 dishes was determined daily, and the average cell number was calculated. On day 8, the growth curve was constructed, and the population DT was calculated by the following formula: $DT = T/x$, where T = total time elapsed and x = number of generations. $\log N = \log N_0 + x \log 2$ where N = final number of cells, N_0 = initial number of cells.

Morphological Studies. For light microscopy, the RETECs were cultured in 75-mm flasks and 60-mm Petri dishes. In some cases, the cells were cultured on glass coverslips (BioCoat, Fort Washington, Pennsylvania), fixed with a mixture of 2% paraformaldehyde and 0.5% Triton X-100 in PBS, and then mounted on glass slides. The cells were examined and photographed with a phase contrast microscope (Zeiss Axiovert 25, Munich, Germany). For scanning electron microscopy (SEM), the cells were cultured on glass coverslips, and after rinsing with PBS, they were fixed overnight with 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer. After washing with cacodylate buffer, the cells were then immersed in a saturated solution of thiocarbohydrazide and incubated in 1% osmium tetroxide. After graded dehydration with ethanol, the specimens were critical point dried with carbon dioxide, coated with gold, and then examined by SEM (Hitachi, S-3200N, Tokyo, Japan). For transmission electron microscopy (TEM), cells were grown on plastic coverslips (Nunc, Naperville, Illinois) or on semipermeable membranes (Costar Corp, Cambridge, Massachusetts). In either case, the cells were fixed with 2.5% glutaraldehyde in 100 mmol/L sodium cacodylate buffer (pH 7.2) for 2 hours at room temperature. After washing in cacodylate buffer, the cells were postfixated in 1% osmium tetroxide for 1 hour and dehydrated in ethanol. The specimens were then embedded in epoxy resin. For

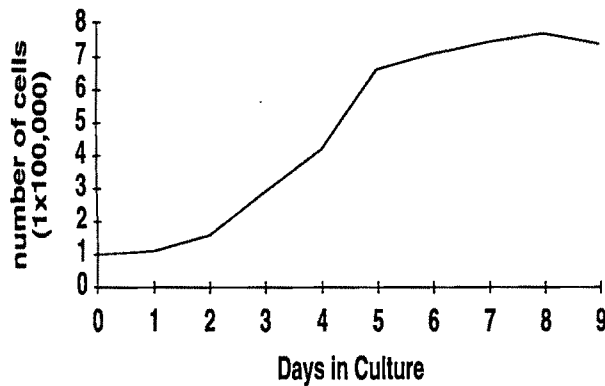


Fig 1. Growth curve of rat eustachian tube epithelial cell line (RETEC). Immortalized rat eustachian tube epithelial cells show strong growth capability.

the cells grown on membrane filters, the filters were cut into small pieces and embedded in flat molds. For the cells grown on coverslips, on the other hand, the coverslips were inverted, cell-side down, onto silicone rubber molds filled with freshly mixed resin. The epoxy resin was cured at 60°C for 24 hours, and the plastic coverslip was removed from the resin block and immersed in liquid nitrogen. Ultrathin sections were prepared and stained with uranyl acetate and lead citrate. The sections were visualized by TEM with a CM120 Biotwin FEI microscope (Philips, Portland, Oregon).

Immunocytochemistry. An indirect immunofluorescence method was used to stain for SV40 T antigen, p53, cytokeratin, and vimentin. Briefly, cells cultured on collagen type I-coated coverslips were fixed with prechilled methanol (−20°C) for 20 minutes, permeabilized with acetone (4°C) for 2 minutes, dried, and then rinsed with PBS. The cells were blocked with 10% normal goat serum for 30 minutes at 37°C. The cells were then incubated with an anti-pancytokeratin antibody, the monoclonal antibody K4.62 (which labels cytokeratin 19), and a monoclonal anti-vimentin antibody¹² (Sigma) for 5 hours at room temperature. After washing, the coverslips were covered with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Oncogene Science) and incubated for 1 hour at 37°C.

For ZO-1 staining, the cells cultured on the coverslips were fixed and permeabilized with a mixture of 2% paraformaldehyde and 0.5% Triton X-100 in PBS for 20 minutes at room temperature. The cells were blocked with a mixture of 3% goat serum and 3% fetal bovine serum in PBS for 30 minutes at 37°C. After washing, the coverslips were incubated with a rabbit anti-ZO-1 antibody (kindly provided by Dr Parviz Farshori, Laboratory of Cellular Biology, National Institute on Deafness and Other Communication Disorders, Bethesda, Maryland) for 1 hour at

CHARACTERISTICS OF RAT EUSTACHIAN TUBE EPITHELIAL CELL LINE

Life span (passages)	>38
Doubling time in log phase (h)	36.92
No. of generations in log phase	2.60
Saturation density (60-mm dish)	7.71×10^5
Cobblestonelike appearance	+
Dome formation	+
Tight junction presence	+
Colony formation in soft agar	−
Vimentin staining	−
Pancytokeratin staining	+
ZO-1 staining	+
Simian virus 40 staining	+
p53 Staining	+

37°C, and then incubated with the anti-rabbit IgG antibody conjugated with FITC for 1 hour at 37°C. Vero cells were used as a negative control. All coverslips were mounted on glass slides with glycerin mounting gel and then observed and photographed with a light microscope equipped with incident-light fluorescence (Zeiss, Axiovert 135).

Colony Formation in Soft Agar. Cell suspensions of the RETEC (5×10^4 cells per milliliter) in 2 mL of 0.36% Noble agar with culture medium were overlaid onto a 60-mm Petri dish containing 5 mL of 0.96% base agar. Human epidermal carcinoma (KB) cells were used as a positive control. The plates were checked after 21 days, and any colony larger than 0.125 mm in diameter was counted.

Western Blotting. The Western blot method of Towbin et al¹³ was used with modification. Confluent cultures of the cells were washed with PBS (pH 7.2) and suspended in a buffer containing 0.1 mol/L sodium chloride and 0.01 mol/L Tris (pH 7.6) with a protease inhibitor cocktail and pepstatin (Boehringer Ingelheim, Biberach, Germany) at 4°C. One volume of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer was added, and the mixture was boiled for 10 minutes before running on a 7.2% SDS-PAGE gel. The proteins were transferred from gels onto nitrocellulose membranes at 250 mV for 3 to 4 hours in Bio-Rad transfer buffer (pH 7.5; Hercules, California). After blocking with 3% skim milk, the membranes were incubated with mouse monoclonal antibodies against SV40 T antigen or p53 for 2 hours and then with alkaline phosphatase conjugated anti-mouse IgG for 1 hour. The membranes were developed in 5-bromo-4-chloro-3-indolyl phosphate–nitro blue tetrazolium salt (BCIP/NBT) substrate solution (Sigma) to visualize the bands. All procedures were performed at room temperature.

Chromosomal Analysis. At passage 18, species-spe-

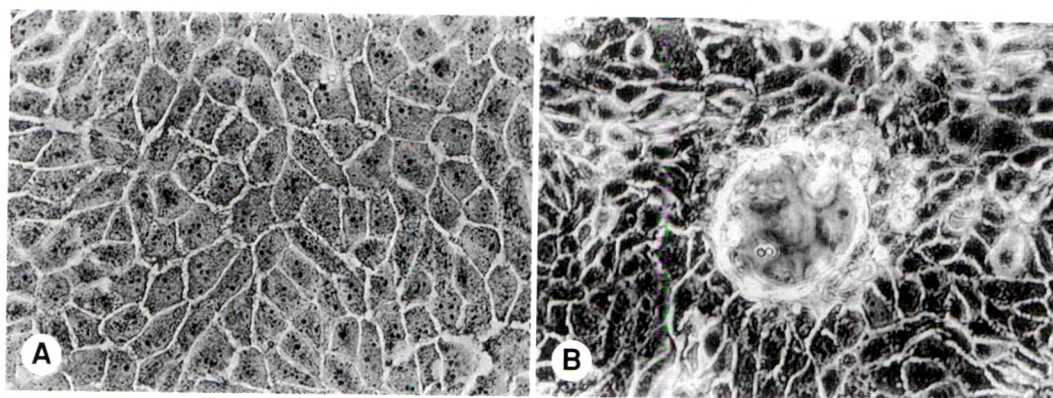


Fig 2. Phase-contrast light micrographs (original $\times 320$). **A)** Passage 18 RETEC, after 11 days in culture, shows cobblestonelike appearance, result of tight junction formation. **B)** Passage 19 RETEC, after 12 days in culture, shows dome formation.

cific enzymatic, cytogenetic, and karyotypic analyses of the RETECs were performed by Dr Bharati

Hukku, Children's Hospital of Michigan, Detroit, Michigan.

RESULTS

Primary Culture and Immortalization. By the third day after explantation, an outgrowth of the epithelial cells was observed in most explants. The cell monolayer was 80% confluent on day 23, when the cultures were infected with the Ad12-SV40 hybrid virus. Six weeks after virus infection, 2 of the 4 virus-infected cultures displayed higher growth. These high-growth cells attained confluence within 1 week and were then subcultured at a 1:2 ratio at passage 4. At passage 10, cells were cloned, and 1 (No. 3) of 7 single-cell clones was selected and cultured further. This virus-infected eustachian tube epithelial cell clone has undergone 38 passages over 10 months, and has been designated Rat Eustachian Tube Epithelial Cell Line (RETEC). Vero cell cultures that were inoculated with the supernatant of RETECs showed no evidence of virally infected or dead cells.

Proliferation and Doubling Time. The growth curve of the RETECs contains obvious lag and log phases, with negligible growth occurring during the lag phase and exponential growth taking place during the log phase (Fig 1). At day 8, the generation number of the RETECs was 2.93, and the average doubling time was 65.75 hours, whereas during the log phase, the generation number and the doubling time were 2.60 and 36.92, respectively (see Table). The RETECs showed a weak negative proliferation after 8 days, and the saturation density of the cells in the 60-mm Petri dish was 7.71×10^5 during the 8-day growth period.

Morphology. When trypsinized and plated on a culture surface, the RETECs were initially spherical, but by about 7 hours, filopodia could be seen. The ends of the filopodia commonly displayed a branched shape and gradually became wide, with ruffled edges. The cells first established contact with one another

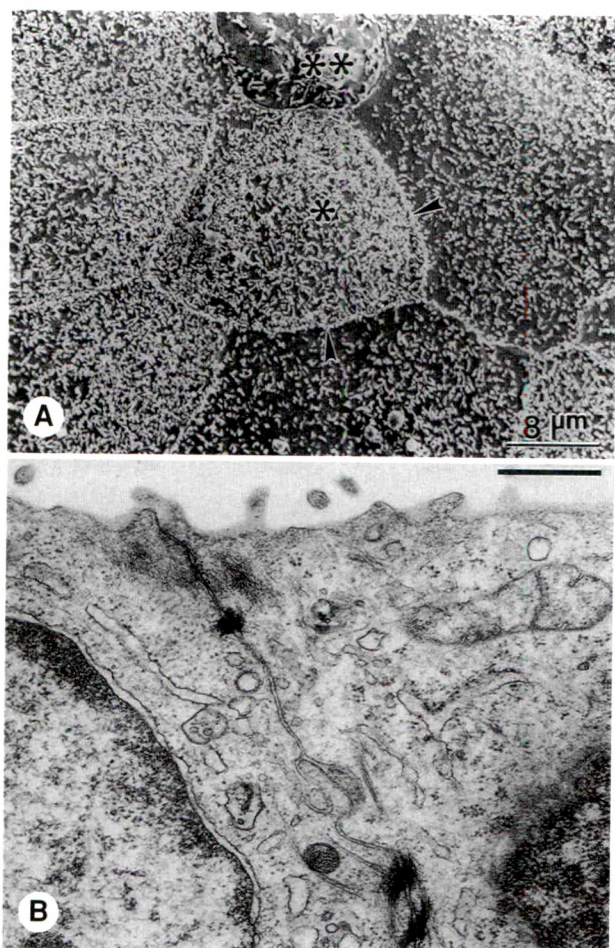


Fig 3. **A)** Scanning electron micrograph of RETECs. Distinct cell margins can be seen between cells (arrowheads), as can numerous microvilli on cell surface. Microvillus density varies from cell to cell (single and double asterisks). **B)** Transmission electron micrograph of RETECs shows that they have retained epithelial cell characteristics such as presence of microvilli on their apical surface and existence of junctional complexes (tight junctions and desmosomes) on their basolateral surface. Scale bar — 1 μ m.

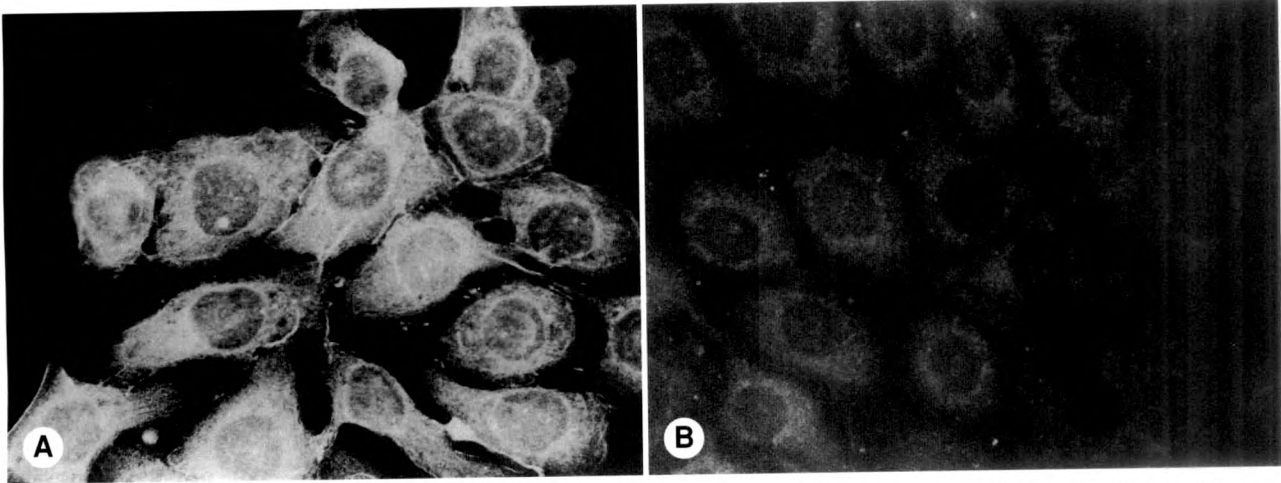


Fig 4. Indirect immunofluorescent labeling of RETECs with anti-cytokeratin and anti-vimentin antibodies. **A)** Positive labeling of RETECs by monospecific anti-cytokeratin antibody K4.62, which reacts with cytokeratin 19. **B)** Negative staining of RETECs by anti-vimentin antibody.

via the filopodial projections, but once confluent, the cells formed tight junctions and took on the typical polygonal cell shape and cobblestonelike appearance (Fig 2A). The monolayers of the RETECs were often punctuated with the "dome" formations¹⁴ that result from the accumulation of fluid in cavities between the epithelial cell monolayer and the surface of the Petri dish (Fig 2B).

Under SEM, the RETECs showed different cell sizes and distinct margins between the cells (Fig 3A). Microvilli, but not cilia, were also observed on the cell surface. The density of the microvilli varied from cell to cell, and the distribution of the microvilli could vary on the surface of one cell. Additionally, TEM studies showed that the microvilli were present on the apical surface of the cells and that junctional complexes (tight junctions and desmosomes) were expressed on their basolateral surface, consistent with their epithelial origin (Fig 3B).

Immunocytochemistry. The RETECs showed posi-

tive staining with an anti-pancytokeratin antibody and the monoclonal antibody, K4.62, which recognizes cytokeratin 19 only. The cells did not stain with anti-vimentin antibody (Fig 4). Staining for ZO-1, a protein found in epithelial cell tight junctions, was also positive in the RETECs (Fig 5), but not in Vero cells (data not shown).

Colony Formation in Soft Agar. After 21 days of growth in soft agar, the RETECs showed no sign of colony formation, whereas the KB epidermal carcinoma cells had formed numerous colonies.

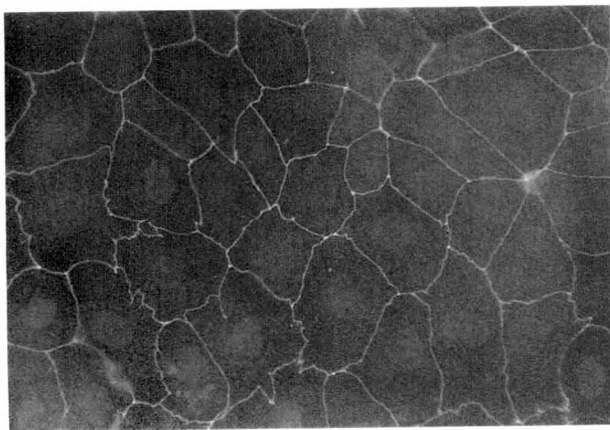


Fig 5. Antibody against ZO-1 labels tight junctions of RETECs and shows that cells have typical polygonal pattern.

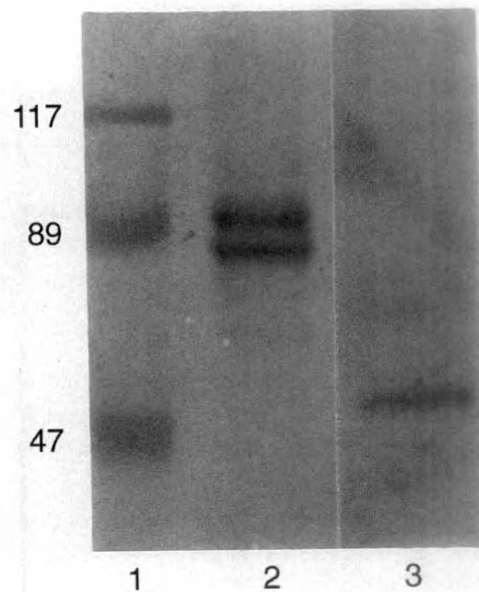


Fig 6. Western blot analysis of RETEC. Lane 1 is molecular weight markers. Lane 2 is approximately 94 kD band detected by antibody against simian virus 40 (SV40) large T antigen. Smaller band may be product of partial degradation of SV40 large T antigen. Lane 3 is approximately 53 kD band detected by antibody against p53.

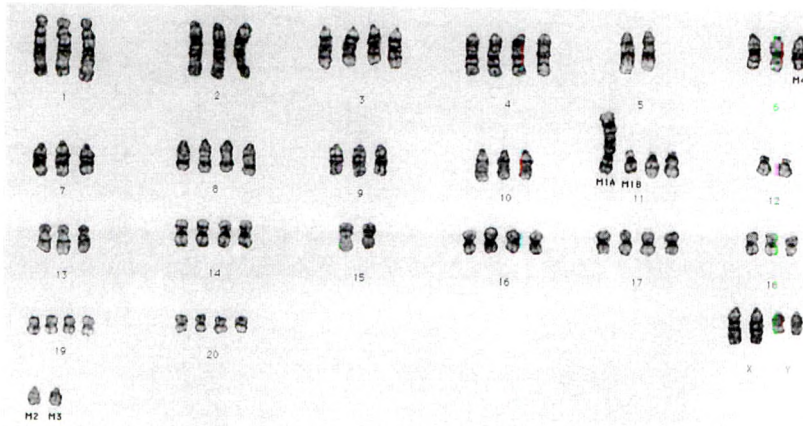


Fig 7. Karyotype analysis. RETECs are of male origin and hypotetraploid, with 93% of chromosome counts in 68 to 77 range ($2N = 42$).

Western Blotting. In Western blots of the immortalized RETECs, a 94 kd band was observed with a monoclonal antibody against SV40 T antigen, and a 53 kd band was seen with a monoclonal antibody against p53 (Fig 6).

Chromosomal Analysis. Chromosomal analysis of the RETECs revealed that the cells were aneuploid and derived from a male rat (XYY/XYY). Most of the chromosome counts reflected hypotetraploidy, with 93% of the counts in the 68 to 77 range ($2N = 42$; Fig 7). All of the cells reacted with fluorescein-conjugated anti-rat antiserum and for 7 rat marker chromosomes. Cells other than those of the cell line were not detected in the culture by cytogenetic evaluation. Isozyme analysis and reactions with antisera proved that there was no contamination with other cell lines.

DISCUSSION

The Ad12-SV40 hybrid virus has been used to immortalize several cell types from various origins.^{9,15-19} The hybrid virus produces messenger RNA from the transforming region of SV40, but not from the early 1 region of adenovirus 12.²⁰ The SV40 T antigen plays an important role in the immortalization process. The binding of SV40 T antigen to the protein product of the tumor suppressor gene p53, which is expressed at high levels in SV40 T antigen transformed cells, appears to be essential for extending the proliferative potential of human fibroblasts.²¹

Typically, the rodent cells isolated from normal tissue will complete a given number of generations in vitro, display a decreased growth potential with increasing passages, and eventually stop growing and die. The RETECs, in contrast, began to display an enhanced growth rate starting at the fourth passage, and have been successfully propagated since that time: 38 passages over 10 months. The growth curve of the RETECs indicates that they have a strong proliferative potential, similar to that seen in immortal-

ized rat⁵ or chinchilla²² middle ear epithelial cells. The presence of a 94 kd band detected by the SV40 T antigen antibody and a 53 kd band detected by the p53 antibody in Western blot analysis is consistent with the expression of SV40 T antigen and p53 by these cells. The cells did not display a fully transformed phenotype or malignant growth,¹⁹ as evidenced by the lack of colony formation in soft agar and the absence of foci in confluent monolayers.^{22,23} Chromosomal analysis revealed that the RETEC line consists of male aneuploid rat cells. These results indicate that the RETEC line has been immortalized by an increase in the expression of the SV40 T antigen and of p53.

Cytokeratin and vimentin are 2 of the 5 major types of intermediate filaments that constitute the cytoskeleton. Cytokeratin, which consists of 19 distinct polypeptides in human epithelial cells, is a specific marker for these cells, whereas vimentin is a marker for cells of mesenchymal origin,^{24,25} such as fibroblasts. Although the expression of cytokeratin in rat middle ear epithelial cells has been reported, there have been no reports demonstrating expression of cytokeratin in rat eustachian tube epithelial cells. For our studies, we used 13 types of cytokeratin antibodies as epithelial markers for RETECs. The immortalized cells were positive for cytokeratin polypeptide 19. An antibody to vimentin, however, did not stain the RETECs, whereas Vero cells were strongly stained by this antibody. The presence of tight junctions in the RETECs was confirmed by staining with an antibody against ZO-1, a protein in tight junctions of mammalian epithelial and endothelial cells.²⁶

Morphologically, RETECs are polygonal in shape, have tight junctions, grow in monolayers, and have a cobblestonelike appearance with numerous microvilli; these findings suggest that this cell line has maintained the differentiated morphological characteristics of cultured epithelial cells. Dome formation by RETECs indicates that they are involved in ion

and water transport and that the monolayer is composed of functioning polarized epithelial cells.^{27,28} Together, these results suggest that the cell line indeed originated from epithelial cells.

In summary, an RETEC line was successfully established by infecting primary cultures of rat eustachian tube epithelial cells with the Ad12-SV40 hy-

brid virus. The cell line retains differentiated characteristics of normal rat epithelial cells, has been in continuous culture for nearly a year, and does not display malignant growth characteristics. This cell line will be useful for studying the normal biology of the eustachian tube epithelial cells, as well as for elucidating the cellular and molecular mechanisms involved in the pathogenesis of otitis media.

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CELLULAR PROLIFERATION OF MUCOSA-ASSOCIATED LYMPHOID TISSUE WITH OTITIS MEDIA: A PRELIMINARY STUDY

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Histopathologic and immunohistochemical analyses were used to investigate the cellular proliferation of mucosa-associated lymphoid tissue (MALT) in human temporal bones with and without evidence of otitis media (OM). Anti-proliferating cell nuclear antigen (PCNA) antibody (clone PC10) was applied after the antigen retrieval procedure. Positive PCNA expression was observed in temporal bones that had been stored for 10 to 31 years in 80% ethanol. In specimens with purulent OM, the MALT had faint germinal centers (GCs). Positive PCNA expression in the MALT was moderate and scattered. In specimens with mucous OM, the MALT had complete GCs. Positive PCNA expression in the MALT was moderate to strong, and the distribution of PCNA-positive cells was associated predominantly in the GCs, the mucosal epithelial layer, and/or the subepithelial layer. In specimens with serous OM, the MALT also had complete GCs. However, the PCNA expression was weak and scattered, and appeared to be similar to that of the MALT in the temporal bones without OM. These results indicate that the cellular proliferation of MALT in the temporal bone might reflect the activity that produces secretory IgA against invasion of foreign antigens. However, further studies are needed to elucidate whether the PCNA expression within MALT in the eustachian tube and middle ear is associated with a mucosal immune response to inflammation as in OM.

KEY WORDS — immunohistochemistry, mucosa-associated lymphoid tissue, otitis media, proliferating cell nuclear antigen, temporal bone.

INTRODUCTION

Otitis media is one of the most common diseases of the middle ear (ME), especially among infants and children. In patients with otitis media (OM), inflammatory cells are observed in the mucosa and the fluid in the ME lumen. Furthermore, mucosa-associated lymphoid tissue (MALT) is found frequently in the ME mucosa and the eustachian tube (ET) mucosa of patients with OM.^{1,2} Matsune et al¹ found that 64% and 21% of children with OM had MALT in the ET and the ME, respectively. Kamimura et al² found that MALT did not appear in the ET of neonates with OM, but that 62% of infants and children with OM had MALT. Additionally, the prevalence of MALT in adults with OM (11%) was lower than that in infants and children statistically.² These studies suggest that MALT has a close relationship to OM and that it may be a local response to repeated infection.

Mucosa-associated lymphoid tissue is characterized histologically by rounded aggregations of lymphocytes in the lamina propria, frequently with germinal centers (GCs). Immunohistologic studies of MALT in temporal bone,³ maxillary sinus,⁴ bronchus,⁵⁻⁷ gut,^{8,9} and larynx¹⁰ have revealed a similar

distribution of lymphocyte subsets. Tsurumaru⁴ also presented evidence that lymphoid follicle formation in the intramaxillary mucosa could change in response to the phase of inflammation. This finding suggests that MALT may change its morphology to respond against antigens (antigen uptake and secretion of IgA). However, little is known about the formation of MALT in the ME and the ET, the proliferative potential of MALT, or how MALT secretes immunoglobulin.

Proliferating cell nuclear antigen (PCNA) is a DNA polymerase- δ -associated auxiliary protein that is involved in DNA synthesis and cellular proliferation.^{11,12} In the field of human temporal bone research, anti-PCNA immunohistochemical staining has been used to investigate cellular proliferation in the inner ear,¹³ the tympanic membrane,¹⁴ cholesteatoma,¹⁵⁻¹⁸ malignant melanoma,¹⁹ and acoustic neuroma.²⁰ Jokay et al¹³ stained formalin-fixed, trichloroacetic acid-decalcified, paraffin-embedded temporal bone specimens removed from fetuses (10 to 14 weeks) with anti-PCNA antibody immunohistologically, and they found PCNA-positive cells in the cochlea. Wiet et al²⁰ investigated PCNA in the

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PCNA EXPRESSION IN MALT WITH AND WITHOUT OTITIS MEDIA

Case	Type of Otitis Media	Age	Cause of Death	Shape of GCs	PCNA Expression*	Distribution of Positive Cells
1	Purulent	1 mo	EEC syndrome	Faint	++	Scattered
2	Purulent	1 y	Congenital heart disease	Faint	++	Scattered
3	Mucous	1 mo	Hydrocephalus	Complete	+++	GCs, E, SE
4	Mucous	5 y	Congenital heart disease	Complete	++	GCs, E, SE
5	Serous	9 mo	Multiple congenital anomalies	Complete	+	Scattered
6		3 mo	SIDS	Complete	+	Scattered

PCNA — proliferating cell nuclear antigen; MALT — mucosa-associated lymphoid tissue; GCs — germinal centers; EEC syndrome — ectrodactyly-ectodermal dysplasia-clefting syndrome; E — epithelium; SE — subepithelium; SIDS — sudden infant death syndrome.
 *+ — weak; ++ — moderate; +++ — strongly positive.

determination of growth rates in acoustic neurinoma and mentioned that the advantage of using the PCNA immunohistochemical technique is that it is useful for archival formalin-fixed, paraffin-embedded tissues. These reports suggested that PCNA immunohistochemical staining is appropriate for archival human temporal bone specimens that have been fixed in formalin, decalcified in trichloroacetic acid, and embedded in celloidin.

This immunohistologic study examined the expression of PCNA by MALT of the ME and the ET in cases of 3 different types of OM (purulent, mucous, and serous) and in 1 control case without OM. The aim of this study was, first, to confirm the feasibility of using anti-PCNA immunohistochemical staining in celloidin-embedded human temporal bone specimens after long-term storage and, second, to investigate the relationship between the type of OM and PCNA expression of MALT and thus to elucidate the function of MALT against invading antigens.

MATERIALS AND METHODS

Celloidin-Embedded Temporal Bone Sections. Temporal bones were obtained at autopsy and fixed in 10% formaldehyde solution. The specimens were decalcified in trichloroacetic acid, dehydrated in graded concentrations of ethanol, embedded in celloidin, and sectioned horizontally or vertically perpendicular to the long axis of the ET at a thickness of 20 to 30 μm .²¹

Specimens for Studying Immunoreactivity With PCNA in Long-Term Stored Celloidin-Embedded Tissue. To examine the expression of PCNA against the lymphoid tissue in celloidin-embedded specimens that have been stored long-term, we used sections of 4 temporal bone specimens from 4 individuals, including lymphoid tissue (MALT in the ME or lymphoid tissue in Rosenmüller's fossa). These temporal bones were obtained at autopsy from 2 infants (both 1 month old) and 2 children (1 and 4 years old). The storage periods were 4, 10, 19, and 31 years.

Specimens for Studying Cellular Proliferation of

MALT With and Without OM. The unstained sections of 6 temporal bone specimens including MALT with and without OM were used. Those specimens were obtained at autopsy from 4 infants (1, 1, 3, and 9 months old) and 2 children (1 and 5 years old): 1 specimen was from a child without OM, 2 specimens were from children with purulent OM, 2 specimens were from children with mucous OM, and 1 specimen was from a child with serous OM. The causes of the children's deaths and the correspondence between their ages and the types of OM are described in the Table. The unstained specimens were preserved in 80% ethanol for 4 to 20 years previous to our immunohistochemical staining.

Histopathologic Staining. Every 10th or 20th section of the temporal bone was stained with hematoxylin and eosin according to our standard technique.

Immunohistologic Staining. Unstained temporal bone sections were soaked in distilled water for 60 minutes and then mounted on silane-coated glass slides. They were incubated in the antigen retrieval solution (saturated sodium hydroxide-methanol solution in methanol at a ratio of 1:3) for 30 minutes at room temperature,²² and rinsed twice in 100% methanol. Endogenous peroxidase was inactivated by incubation with 0.3% hydrogen peroxidase in 90% methanol. The temporal bone specimens were then rinsed in 70% methanol and 45 mmol/L phosphate-buffered saline solution (PBS, pH 7.3), respectively. Microwave antigen retrieval incubation was performed with 10 mmol/L ethylenediaminetetraacetic acid (pH 8.0, Sigma, St Louis, Missouri) in a microwave oven (H2500, Energy Beam Science, Agawan, Massachusetts) at 60°C for 5 minutes.^{3,23,24} Nonspecific antigen binding was inhibited by incubation with blocking solution containing 2% nonfat dry milk, 2% normal goat serum, and 0.1% Triton X-100 (Sigma) for 1 hour at room temperature. Biotinylated monoclonal mouse antibody against PCNA (clone PC10) was obtained from Zymed Laboratories Inc (South San Francisco, California) and diluted to 1:20 with

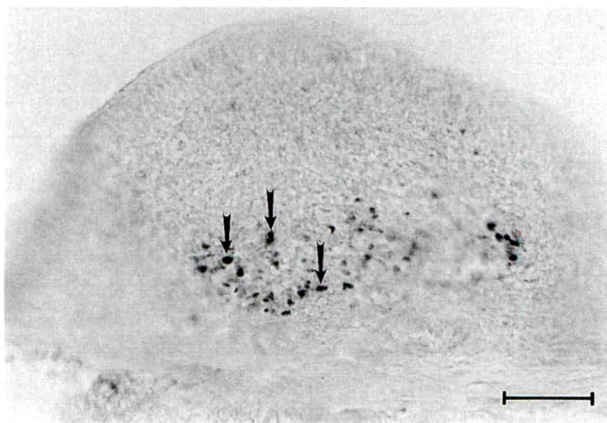


Fig 1. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) in mucosa-associated lymphoid tissue (MALT) of temporal bone stored in 80% ethanol for 31 years. Positive PCNA expression can be observed in germinal centers of MALT in middle ear mucosa (arrows). Bar — 50 μ m.

PBS containing 1% bovine serum albumin (BSA; Sigma) and 0.1% Triton X-100. The sections were incubated with the anti-PCNA antibody at 4°C overnight. After rinsing in PBS, the sections were incubated with avidin-biotin-peroxidase complex (ABC; Vector Laboratories Inc, Burlingame, California) for 1 hour. The color was developed with 0.02% 3,3'-diaminobenzidine•4-hydrochloric acid in 4% sodium

acetate, plus 0.003% hydrogen peroxide. The sections were dehydrated in a graded series of ethanol (70%, 80%, 95%, and 100%), and then immersed in xylene for 15 minutes twice. Finally, these sections were coverslipped and examined under a light microscope.

Normal goat serum was substituted for the antibody as a negative control; celloidin-embedded lymphoid tissue in Rosenmüller's fossa served as a positive control.

Criteria for Identifying OM. All cases had MALT in the ET or ME. Evidence of OM was defined as the presence of histopathologically confirmed inflammatory cells and/or effusion in the ME. This histopathologic definition of OM includes cases of "silent OM" that have histopathologic evidence of ME inflammation but no clinical signs such as otorrhea or perforation.²⁵ Otitis media was further defined by histopathologic conformation as purulent OM (numerous polymorphonuclear leukocytes in the effusion), mucous OM (granular mucosa and thick eosinophilic fluid including chronic inflammatory cells and mucous strands), or serous OM (homogeneous eosinophilic fluid containing few inflammatory cells).²⁶ We defined normal (non-OM) cases by the absence of histopathologically identified inflamma-

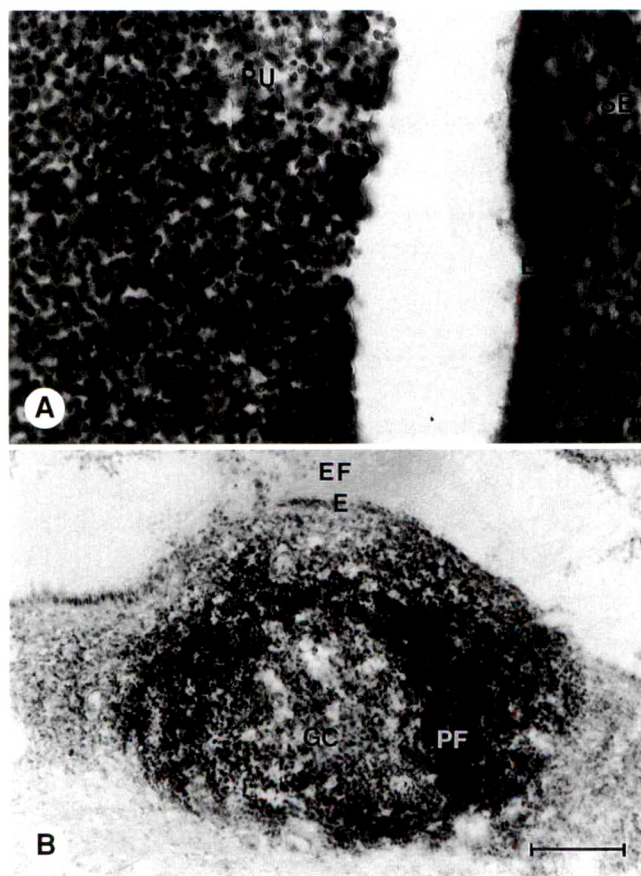


Fig 2. Purulent otitis media case. **A)** H & E staining of middle ear. There is purulent effusion (PU), predominantly containing neutrophils in tympanic cavity. Bar — 50 μ m; E — mucosal epithelium; SE — subepithelium infiltrated by inflammatory cells. **B)** H & E staining of MALT. MALT has faint germinal centers (GCs). Border between GCs and parafollicular area (PF) is ill defined. Bar — 100 μ m; EF — effusion. **C)** Immunohistochemical staining of MALT for PCNA. PCNA-positive reaction is moderate. Distribution of positive cells is scattered, but predominantly in GCs (arrows) and mucosal epithelium (arrowhead). Bar — 100 μ m.

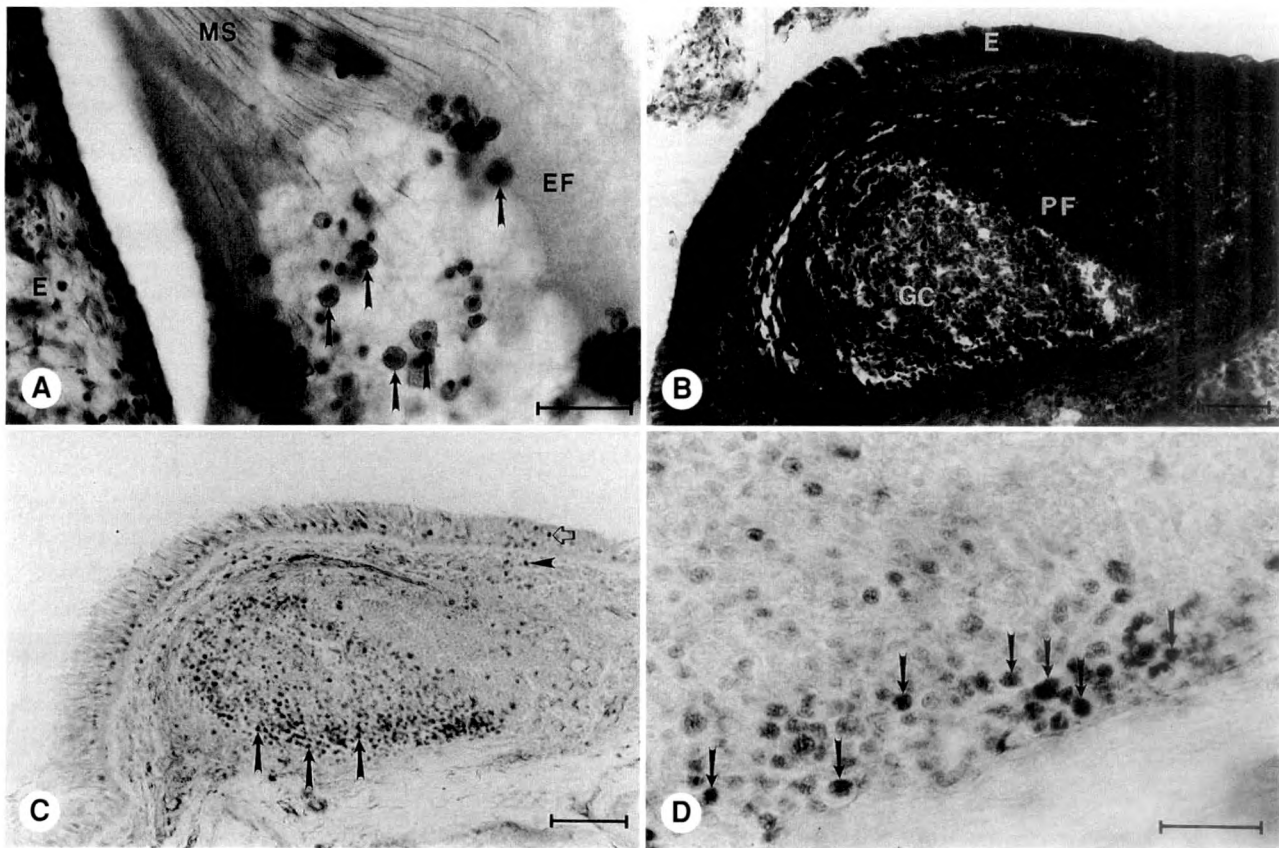


Fig 3. Mucous otitis media case. **A)** H & E staining of mastoid bone. There is thick fluid that contains mucous strands (MS) and chronic inflammatory cells including plasma cells (arrows) and lymphocytes. Bar — 50 μ m; E — epithelium; EF — effusion. **B)** H & E staining of MALT. MALT forms complete germinal centers (GCs) surrounded by parafollicular area (PF). Bar — 100 μ m. **C)** Immunohistochemical staining of MALT for PCNA. Strong positive PCNA expression is observed. PCNA-positive cells are predominantly in GCs (black arrows), in addition to mucosal epithelium (open arrow) and subepithelium (arrowhead). Bar — 100 μ m. **D)** High-magnification photograph of GC with anti-PCNA immunohistologic staining. Lymphocytes show strongly positive PCNA expression (arrows). Bar — 25 μ m.

tory cell infiltration in the ME mucosa and ME cavity. These definitions were adopted from previous articles.^{2,3}

RESULTS

Immunoreactivity for anti-PCNA antibody was observed in all specimens of lymphoid tissue that had been stored in 80% ethanol, even in 4 specimens stored for 4 to 31 years (Fig 1).

PCNA Expression in MALT With and Without OM.

In the temporal bones with purulent OM (Fig 2A), the MALT had poorly differentiated GCs (Fig 2B). Lymphocytes displaying moderate PCNA expression were found throughout the MALT, but were concentrated near the base of the GCs (Fig 2C). In the temporal bones with mucoid OM (Fig 3A), the MALT had well-differentiated GCs (Fig 3B). Lymphocytes displayed moderate to strong PCNA activity. The distribution of PCNA-positive cells was associated predominantly with the GCs, especially the base of the GCs (Fig 3C,D). Additionally, there were some PCNA-positive cells invading the epithelium and/or

subepithelial layer just above the MALT. In the temporal bone with serous OM (Fig 4A), the MALT had some complete GCs and revealed weakly positive PCNA expression. The distribution of PCNA-positive cells was scattered (Fig 4B,C). In the temporal bone without OM, the MALT had complete GCs and revealed weakly positive PCNA expression. The distribution of PCNA-positive cells in the MALT was scattered (Fig 5). These results are summarized in the Table.

Controls. There was a strongly positive reaction of PCNA in the celloidin-embedded lymphoid tissue of Rosenmüller's fossa stained as the positive control. The distribution of PCNA-positive cells was diffuse. On the other hand, there were no stained cells observed in the negative control tissue.

DISCUSSION

In the present study, we were able to recognize positive PCNA expression after the antigen retrieval procedure in temporal bones that had been stored in 80% ethanol for up to 31 years. These temporal bone

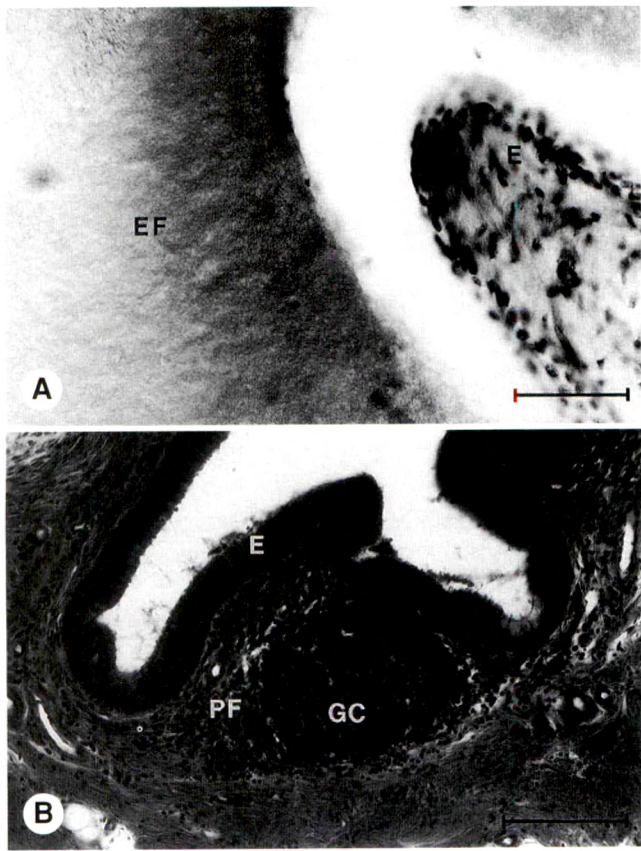
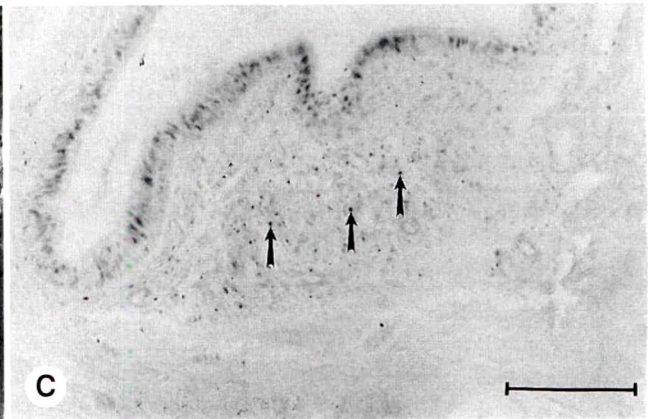


Fig 4. Serous otitis media case. **A)** H & E staining of mastoid bone. There is homogeneous acellular effusion (EF) in tympanic cavity. Bar — 50 μ m; E — mucosal epithelium. **B)** H & E staining of MALT. MALT has complete germinal centers (GCs). Bar — 100 μ m; PF — parafollicular area. **C)** Immunohistochemical staining of MALT for PCNA. Positive PCNA expression is weak. Distribution of positive cells is scattered (arrows). Bar — 100 μ m.



specimens were immersed in trichloroacetic acid for decalcification. Jokay et al¹³ performed an anti-PCNA immunohistochemical study on human temporal bones decalcified in trichloroacetic acid and reported PCNA-positive cells in the fetal cochlea. Thus, unlike that for other proteins,²⁷ the immunohistochemical reaction for PCNA seems to be insensitive to acid treatment. Wolf and Dittrich²⁸ reported previously that the clone PC10 anti-PCNA antibody (used in our study) was superior to other anti-PCNA antibodies (clones 19A2 and 19F4) in recognizing the pro-

tein after prolonged formaldehyde fixation. Our results indicate that the clone PC10 anti-PCNA is also suitable for studies of lymphoid tissue in archival temporal bones.

The role of MALT in the common mucosal immune system (CMIS) has been elucidated gradually during the past decade. The MALT has been divided into 2 types: 1) nasopharyngeal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT), and 2) gut-associated lymphoid tissue

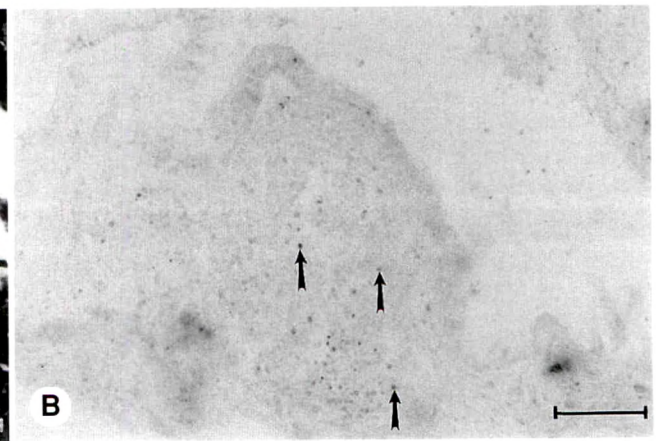
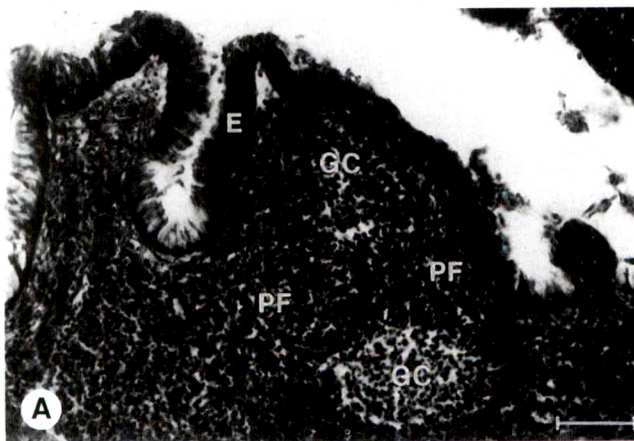


Fig 5. Non-otitis media case. **A)** H & E staining of MALT. MALT forms germinal centers (GCs). Bar — 100 μ m; E — mucosal epithelium; PF — parafollicular area. **B)** Immunohistochemical staining of MALT for PCNA. Positive PCNA expression is weak. Distribution of positive cells is scattered (arrows). Bar — 100 μ m.

(GALT). In the CMIS, induction and proliferation of IgA-producing cells are most important for immune responses against foreign antigens. For example, Peyer's patches and the appendix play a role in GALT, which is considered a major IgA inductive site.²⁹ The GALT includes T cells, B cells bearing surface IgA (sIgA+), macrophages, and dendritic cells.²⁹ A remarkable characteristic of GALT is the presence of a unique epithelium that covers the dome region, composed of follicular-associated epithelial cells (FAE cells)³⁰ or microfold cells (M cells).³¹ Since this epithelium has pinocytotic and phagocytotic activity, it is possible that FAE cells or M cells can act as epithelial antigen-presenting cells.²⁹ The BALT^{6,7} and MALT in the ET³ have a number of morphological similarities to GALT.⁹

The mechanism of homing of secretory IgA (S-IgA)-producing plasma cells coming from GALT to mucosal tissues has been proposed as follows. The sIgA+ B cells leave the inductive site in GALT and enter the blood circulation. The sIgA+ B cells then home to distant mucosal tissues and differentiate into S-IgA-producing plasma cells.²⁹ On the other hand, Tsurumaru⁴ observed only a few IgA-positive cells in the GCs of MALT in the maxillary sinus, but many IgA-positive cells between the mucosal epithelium and MALT. This evidence suggests the possibility that IgA-producing plasma cells might be differentiated from precursor cells in MALT and enter the sub-epithelium directly. Thus, the S-IgA-producing cells from MALT in the maxillary sinus and those from GALT differ in their homing mechanisms.

The GALT and MALT also appear to differ in their time course of formation. From 45 to 100 Peyer's patches appear during gestation, and their numbers reach a maximum at puberty (approximately 240) and decline slowly with age.²⁹ By contrast, MALT is not observed in normal maxillary sinus mucous membranes; rather, they appear during the early stage of chronic inflammation.⁴ Kamimura et al² reported that MALT in the ET and ME was not observed in neonates within 1 month after birth, even though they had OM. The prevalence of MALT in the ET and ME rose 1 month after birth, and the highest prevalence was seen between 1 month and 7 years of age.

In this respect, the MALT in the ET, ME, and maxillary sinus appears to be a response to chronic inflammation. The GALT in Peyer's patches, though, appears to form in the absence of chronic inflammation.

In the present study, the temporal bones with mucous OM had MALT with prominent GCs and showed moderate to strong PCNA expression. These findings suggest that DNA synthesis and cellular proliferation occurred for S-IgA production in the MALT. Moreover, PCNA-positive cells were also observed in the epithelial and/or subepithelial layers just above the MALT. Hence, it is possible that the source of S-IgA-producing plasma cells in the epithelial and subepithelial layers was precursor IgA B cells in the MALT, and that the precursor IgA B cells entered the mucous epithelium from the MALT directly after maturation. This sequence of events would parallel the response in sinus mucosa.⁴

In the single temporal bone with serous OM, there were scattered PCNA-positive cells in MALT that had well-developed GCs. In fact, this MALT was similar to that in cases without OM. These results suggest that there were fewer precursor IgA B cells differentiating to S-IgA-producing plasma cells than in the cases of mucous OM. According to Mogi,³² the mean value of S-IgA in the effusion of serous OM was much smaller than that in mucous OM.

In the temporal bones with purulent OM, the MALT had faint GCs. The positive PCNA expression in the MALT was stronger than that in temporal bones with serous OM and without OM, but weaker than that in temporal bones with mucous OM. These results suggest that purulent OM is an early stage of OM. Persistent and asymptomatic ME effusions are observed in 70% and 20% of children 2 weeks and 2 months after onset of acute OM with purulent effusion, respectively.³³ There is a possibility that if this purulent OM changes to mucous OM, the MALT may form GCs with strong positive PCNA expression for S-IgA secretion. On the other hand, if this purulent OM proceeds to serous OM, the MALT may have GCs with only weak and scattered PCNA expression. However, further studies are necessary to test this hypothesis.

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PROSPECTIVE STUDY ON THE INCIDENCE OF CHRONIC EAR COMPLAINTS RELATED TO GASTROESOPHAGEAL REFLUX AND ON THE OUTCOME OF ANTIREFLUX THERAPY

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Over a 2-year period (1997 to 1999), 5 consecutive adult patients with chronic refractory secretory otitis media (CSOM) and 16 with a chronic refractory feeling of pressure in the ear(s) (CRFP) thought to be related to concomitant eustachian tube dysfunction were prospectively studied for coexisting gastroesophageal reflux (GER). All patients underwent an extensive standardized otorhinolaryngological examination, ambulatory 24-hour dual-probe esophageal pH monitoring with a distal pH probe 5 cm and a proximal probe 20 cm above the lower esophageal sphincter, and upper gastrointestinal endoscopy. Most of them also underwent esophageal manometry. All patients with CSOM and 12 of the 16 patients with CRFP had evidence of GER. Only 5 patients experienced heartburn or regurgitation. All patients responded very well to antireflux therapy with omeprazole 20 mg twice per day (40 mg twice per day in 2 patients) accompanied by conservative antireflux measures, ie, complete cessation of their middle ear complaints. This study demonstrates the role of GER in the pathogenesis of refractory CSOM and CRFP and the effectiveness of sustained antireflux therapy.

KEY WORDS — chronic secretory otitis media, extraesophageal reflux, gastroesophageal reflux.

Gastroesophageal reflux (GER) has been defined as the occurrence of lesions or signs and symptoms secondary to the reflux of gastric contents into the esophagus or beyond into the oral cavity or airways. Lesions attributed to GER include esophagitis, inflammation of the larynx and oropharynx, and acute or chronic pulmonary injury.¹ For the otorhinolaryngologist, GER presents itself with signs and symptoms due to extraesophageal reflux (EER), such as reflux laryngitis and posterior laryngitis with hoarseness, frequent throat clearing, sore throat, globus pharyngeus, and chronic cough.²⁻⁴ Other possible manifestations of EER include laryngospasm,⁵ contact ulcers,^{6,7} laryngeal granulomas,⁸⁻¹⁰ laryngeal and subglottic stenosis,^{2,11} and even laryngeal carcinoma.^{2,12}

Studies using combined esophageal and pharyngeal pH probes demonstrated acid reflux into the pharynx in many of these patients.^{2,13} The important contribution of GER in pediatric inflammatory upper airway disorders has been shown.¹⁴ The term gastropharyngeal reflux has been used to indicate a possible relationship between reflux and pediatric chronic sinusitis.¹⁵

Recently, we encountered a patient with a chronic therapy-resistant ear problem that only resolved after his coexisting GER was diagnosed to be the cause

and consequently was treated successfully as such. This observation suggested to us that some cases of middle ear disease might be considered as another extraesophageal manifestation of GER. In order to learn the prevalence of this association and its clinical properties, we undertook a prospective study to investigate the relationship between chronic middle ear problems and GER. We studied the prevalence of GER in these patients by endoscopy and pH-metry. We also assessed short- and long-term responses to medical antireflux therapy.

MATERIALS AND METHODS

From November 1997 to November 1999, all consecutive patients with predominant complaints of a chronic refractory discharging middle ear (chronic refractory secretory otitis media; CSOM) were referred to our department of gastroenterology. Also referred were all patients with a chronic refractory feeling of pressure (CRFP) in one or both ears thought to be related to eustachian tube dysfunction, with or without manifest changes on tympanometry, but with short-term alleviation of the feeling of pressure after a Politzer or Valsalva maneuver.

These patients were some of the 217 otorhinolaryngology (ORL) patients with chronic complaints thought to be EER manifestations who were referred

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TABLE 1. GROUP WITH CHRONIC REFRACTORY SECRETORY OTITIS MEDIA

Patient No.	Age (y)	Sex	Surgical History	Ear		Additional		UGIT Endoscopy		24-h Double pH Probe		Esophageal Manometry		Omeprazole Maintenance (mg/d)
				Complaints	Signs	Complaints	Signs	Esophagitis Grade	HH	Distal*	Proximal†	LESP	Mot	
1	72	M	Ty L	CD L	Gr	None	Ph	3	None	18.9	11.6	NI	DS	80
2	34	M		CD bilat	Gr, At	None	Ph, PL	1	None	17.5	13.4	NI	NI	20
3	71	F	Ty L, Rad L	CD L	Rad, At	Hr, ST, UC, Ho, GP	Ph	2	None	18.0	19.8	NI	NI	40
4	29	M	NS, ESS	CD bilat	Gr, At	ST, Ho, FO	Ph, PL	2	3 cm	5.6	2.4	7 mm Hg	PP	40
5	57	F	Ty R	CD bilat	Gr	None	Ph, PL	2	None	PF	PF	NI	NI	40

UGIT — upper gastrointestinal tract, HH — hiatus hernia, LESP — lower esophageal sphincter pressure (normal, <10 mm Hg), Mot — esophageal motility, Ty — tympanoplasty, CD — chronic middle ear discharge, Gr — ear granulation(s) and/or polyps, Ph — pharyngitis, NI — normal, DS — diffuse esophageal spasm, bilat — bilateral, At — atelectasis of middle ear, PL — posterior laryngitis, Rad — radical cavity, Hr — heartburn or regurgitation (see text), ST — sore throat, UC — unproductive cough, Ho — hoarseness, GP — globus pharyngeus, NS — nasal septal surgery, ESS — endoscopic sinus surgery, FO — fetor ex ore, PP — poor peristaltic amplitudes (<30 mm Hg), PF — probe failure.

*For distal probe, result of 24-hour dual-probe measurements in percentage of time pH was <4 (see text).

†For proximal probe, result of 24-hour dual-probe measurements in percentage of time pH was <0.8 (see text).

to the department of gastroenterology within this same 2-year period.

The CSOM group consisted of 5 patients with long-standing complaints of a chronic discharging middle ear, unilaterally or bilaterally with tympanic membrane perforations, atelectatic middle ears, and granulations or polyps in the middle ear(s), but without evidence of active cholesteatoma. The cases are called refractory because previous conventional topical and systemic medical therapy and/or surgical treatment(s), including tympanoplasty, mastoidectomy, and nasal and endoscopic sinus surgery, had been of no avail (Table 1). The CRFP group consisted of 16 consecutive patients with chronic refractory complaints of pressure or fullness in one or both ears, thought to be related to concomitant eustachian tube dysfunction (Table 2). All of them had intact eardrums without signs of middle ear effusion. Most of them had no or only minor changes on tympanometry or pure tone audiometry. All of these patients could alleviate their continuous feeling of ear pressure with a repetitive and forceful Valsalva maneuver, but only for some minutes. Other causes for their complaints had been ruled out. Previous conventional medical treatments including antihistamines, nose and ear drops, antibiotics and topical or systemic corticosteroids had only led to short-term or incomplete improvement or no improvement at all.

In order to establish a diagnosis of GER, we submitted all patients to upper gastrointestinal tract endoscopy during which the presence of erosive esophagitis was noted and its degree was scored (1 to 4) according to the classification of Savary and Miller.¹⁶ In addition, ambulatory 24-hour dual-probe esophageal pH monitoring was performed to quan-

tify esophageal acid exposure. The distal probe was located 5 cm above the level of the lower esophageal sphincter (LES). The proximal probe was located 20 cm above the LES, just below the upper esophageal sphincter (UES). Finally, most patients also had esophageal manometry by a stationary pull-through technique to quantify LES pressure and to detect abnormalities in primary esophageal peristalsis.

The diagnosis of GER was made on the basis of the presence of esophagitis and/or a distal acid exposure time (percentage of total time pH was <4) exceeding 4% of the time. Proximal acid exposure time was judged abnormal when it exceeded 0.8%.¹⁷ All patients were screened for other possible reflux-related symptoms, and all underwent an extensive standardized ORL examination including magnifying 90° telescopic laryngoscopy.

All patients with a diagnosis of GER received anti-reflux therapy consisting of omeprazole 20 mg twice per day (2 patients initially took 40 mg twice per day; patients 1 and 4 on Table 1) regardless of endoscopic and pH-metric findings. In addition, they were instructed to use conservative antireflux measures, ie, raising of the head of the bed by 20 to 25 cm, avoiding food and liquids 3 hours before retiring, and other dietary and lifestyle modifications. All patients were followed at 2-week intervals until their ear symptoms had resolved completely. The omeprazole therapy was gradually decreased if the patient was asymptomatic for at least 4 weeks on a given dose. This reduction allowed determination of the lowest effective maintenance dose, if any (Tables 1 and 2). Subsequently, the patients entered long-term follow-up (range, 4 months to 2 years; mean, 11 months).

TABLE 2. GROUP WITH CHRONIC REFRACTORY FEELING OF PRESSURE IN EAR

Patient No.	Age (y)	Sex	Surgical History	Ear		Additional		UGIT Endoscopy		24-h Double pH Probe		Esophageal Manometry		Omeprazole Maintenance (mg/d)
				Complaints	Signs	Complaints	Signs	Esophagitis Grade	HH	Distal*	Proximal†	LESF	Mot	
1	67	F		FP bilat		None	Ph, PL	0	None	6.9	3.1	NI	NI	20
2	75	F		FP bilat		UC, Ho	Ph	0	2 cm	5.7	0.5	NI	NI	20
3	55	M	Ty 2x L, VT	FP L	RP	None	PL	1	None	3.0	0.0	NI	NI	20
4	56	M		FP bilat		Hr, LS, ST, UC, Ho	Ph, PL	2, Ba	4 cm	21.0	2.6	0 mm Hg	PP	40
5	29	F	ESS, CC	FP bilat		Hr, ST, UC, Ho, FO	Ph, PL	1	None	0.4	0.0	NI	NI	20
6	70	F		FP bilat		LS, ST, UC, Ho	PL	0	None	13.2	3.0	NI	NI	20
7	39	F		FP bilat		ST, Ho	Ph, PL	0	None	3.0	0.3	NI	NI	None
8	54	F		FP L		ST	Ph, PL	0	None	2.3	1.7	NI	DS	None
9	27	M		FP bilat		ST, GP	PL	0	None	0.1	0.0	NI	NI	None
10	31	F	ESS	FP L		ST	Ph, PL	1, GU	3 cm	1.8	0.8	NR	NR	20
11	58	M	CC	FP bilat		LS, ST, FO	Ph, PL	2	3 cm	12.0	2.4	NI	DS	40
12	68	M		FP bilat		Hr, LS, UC, Ho	Ph, PL	2	3 cm	PF	PF	NI	NI	20
13	42	M	VT	FP bilat		None	Ph	0	None	0.6	0.0	8 mm Hg	NI	20
14	68	F		FP L		ST	Ph	0	None	4.3	3.7	NI	NI	None
15	47	M	CL	FP R, EP		Hr, LS, ST, UC, Ho, FO	Ph, PL	1	3 cm	8.1	3.3	NI	DS	40
16	66	M		FP bilat		ST, FO	Ph, PL	2, Ba	None	9.0	0.7	8 mm Hg	NI	20

UGIT — upper gastrointestinal tract, HH — hiatus hernia, LESF — lower esophageal sphincter pressure (normal, <10 mm Hg), Mot — esophageal motility, FP — feeling of fullness and/or pressure, bilat — bilateral; Ph — pharyngitis, PL — posterior laryngitis, NI — normal, UC — unproductive cough, Ho — hoarseness, Ty — tympanoplasty, VT — ventilation tube, RP — retraction pocket, Hr — heartburn, LS — laryngeal spasm, ST — sore throat, UC — unproductive cough, Ba — Barrett's esophagus, PP — poor peristaltic amplitudes (<30 mm Hg), ESS — endoscopic sinus surgery, CC — conchal cauterization, FO — fotor ex ore, DS — diffuse esophageal spasm, GP — globus pharyngeus, GU — gastric ulcer, NR — no results, PF — probe failure, CL — Caldwell Luc procedure, EP — ear pain.

*For distal probe, result of 24-hour dual-probe measurements in percentage of time pH was <4 (see text).

†For proximal probe, result of 24-hour dual-probe measurements in percentage of time pH was <0.8 (see text).

RESULTS

From November 1997 to November 1999, all 217 consecutive ORL patients with chronic symptoms thought to be caused by GER were referred to the department of gastroenterology for investigation and subsequently received antireflux treatment. The predominant complaints were chronic sore throat, unproductive cough, hoarseness, globus pharyngeus, and laryngospasm.

Among the patients were 21 with predominant chronic ear complaints; 5 of them had CSOM and 16 had CRFP. All patients with CSOM (Table 1) and 12 patients of the 16 with CRFP (Table 2) received a diagnosis of GER.

Erosive esophagitis was found in 13 patients (62%), and was found in 100% of the CSOM patients — usually to a more severe degree in them than in the CRFP patients, of whom 50% had erosive esophagi-

tis. Hiatus hernia was found in 7 patients (33%). We also found 2 patients with Barrett's esophagus and another patient with a gastric ulcer (*Helicobacter pylori*-negative).

Distal acid exposure was pathological in 12 patients (64%); proximal acid exposure was pathological in 11 patients (58%). The CSOM patients had highly pathological values of distal acid exposure (mean, 15%; range, 5.6% to 18.9%) and of proximal acid exposure (mean, 11.8%; range, 2.4% to 19.8%). In the CRFP group, distal acid exposure (mean, 6%; range, 0.1% to 21%) was pathological in 8 patients (53%), and proximal exposure (mean, 1.5%; range, 0% to 3.7%) was pathological in 7 patients (47%).

Esophageal manometry showed poor peristaltic amplitudes (<30 mm Hg) in 2 patients and intermittent high-amplitude simultaneous contractions indicative of diffuse esophageal spasm in 4 other patients.

Four patients had a low resting LES pressure of <10 mm Hg. Thus, overall, an esophageal motility disorder and/or a too-low LES pressure (<10 mm Hg) was found in 8 patients (40%).

Other ORL symptoms related to chronic reflux were a sore throat (13 patients or 62%), hoarseness (9 or 43%), unproductive cough (7 or 33%), and laryngospasm (5 or 24%). Only 5 patients (24%) experienced typical heartburn or regurgitation.

The chronic reflux-related ORL signs were marked erythema of the pharynx (pharyngitis) in 18 patients (86%) and marked erythema and/or edema of the posterior larynx (posterior laryngitis) in 16 patients (76%). There were no significant differences between the CSOM and the CRFP groups in the occurrence of additional symptoms, nor in the findings of posterior laryngitis and pharyngitis. Of the CSOM group, 3 of the 5 patients had bilateral ear disease, and of the CRFP group, 11 did (69%). Of the CSOM patients, 3 had a history of ear surgery. Of the CRFP patients, only 1 had a history of a failed tympanoplasty.

On antireflux therapy with omeprazole 20 mg twice per day (2 patients took 40 mg twice per day: patients 1 and 4 on Table 1), the middle ear discharge completely ceased in all CSOM patients, and the middle ear granulations and/or polyps disappeared, leading to a residual dry perforation or a dry atelectatic middle ear. The average time this resolution took was 11 weeks (range, 6 to 16 weeks). One patient (patient 4 on Table 1) discontinued his treatment on his own initiative; his original problems reappeared, to heal again only when his therapy was restarted. At the end of follow-up, the CSOM patients remained on a median maintenance dose of 40 mg of omeprazole per day. All CRFP patients became symptom-free after an average time of 4 weeks (range, 2 to 6 weeks) with antireflux therapy and omeprazole 20 mg twice per day. This reaction to therapy was also noted in the 4 patients who did not have GER according to the above-mentioned criteria (patients 7, 8, 9, and 13 on Table 2); they were nevertheless included in this series because of their complaints and the signs found on clinical examination.

The tympanograms that were available on follow-up (6 patients, of whom 3 initially had type C curves) normalized simultaneously with the disappearance of the symptoms.

At the end of follow-up, the CRFP patients remained on a median maintenance dose of 20 mg of omeprazole per day ($p < .01$ as compared to CSOM patients). The ORL examination revealed that after the patients became symptom-free, the erythema of

the pharynx and the posterior larynx disappeared as well. The additional symptoms, if present, also responded to therapy.

DISCUSSION

Reflux may be assessed by a variety of diagnostic tests. If, on upper gastrointestinal tract endoscopy, erosive esophagitis or Barrett's esophagus is found, a GER diagnosis is established. The use of upper gastrointestinal tract endoscopy has not been routinely advocated in ORL patients, because they usually do not experience heartburn or regurgitation (less than half of patients, in most studies) and because erosive esophagitis in these patients is considered to be a rare finding. Large prospective studies underscoring this policy, however, are lacking.

Ambulatory 24-hour pH monitoring is considered to be ideal in ORL patients suspected of having GER. Dual-probe pH monitoring with one electrode in the distal esophagus, 5 cm above the LES, and a second probe 20 cm above the LES in the proximal esophagus just below the UES is considered preferable.¹ The major advantage is the possibility of quantifying distal and proximal acid exposure times on which normal values are available, making this a useful standard.^{17,18} If abnormal distal esophageal acid exposure is found, the diagnosis of GER is established.

Documentation of abnormal proximal esophageal acid exposure suggests a higher potential for acid reflux to reach the laryngopharynx and the upper airways, and therefore the probability becomes higher that the ORL symptom or sign is due to GER.¹ Furthermore, abnormal proximal reflux may predict the response to medical therapy in patients with pulmonary disease,¹⁹ but this correlation is less clear in ORL patients. Probes placed in the hypopharynx can document reflux above the UES, giving more certainty to laryngopharyngeal reflux as the cause of ORL symptoms or signs. This placement of the probe above the UES would be ideal if there were not a number of technical problems (including possible interpretation errors due to probe drying and other artifacts resulting in a drop of pH to <4 that is not a true reflux episode). At present, normal values are not available.¹ Esophageal manometry can demonstrate an esophageal motility disorder, including a resting pressure in the LES that is too low, which may also contribute to ORL problems due to GER.

The results of our first 75 patients were reported previously.²⁰ In the majority of that group (77%), GER could be demonstrated, and treatment with a proton pump inhibitor induced relief of symptoms within 4 weeks in most of them (82%).

Our data suggest that GER sometimes may be di-

agnosed in patients with chronic ear complaints thought to be related to eustachian tube dysfunction and in patients with CSOM. Reflux of gastric contents (ie, acid and pepsin) into and beyond the esophagus and into the pharynx and nasopharynx might lead to eustachian tube dysfunction, tubotympanitis, and CSOM through an inflammatory reaction and possibly secondary infection. Erosive esophagitis and abnormal distal and proximal esophageal acid exposure on 24-hour pH monitoring were not only more prevalent, but usually also more serious, in the CSOM patients than in the CRFP patients — a finding suggesting a direct relationship between the severity of ear problems and of GER in some of the CSOM patients. This seems to be confirmed by the longer treatment period required to obtain symptom relief in CSOM patients (11 weeks) than in CRFP patients (4 weeks). It is also supported by the higher dose of omeprazole needed to keep the CSOM symptoms under control after the initial treatment. It might be reasoned that most of the CRFP patients have no real ear disease, since no abnormalities were found on otoscopy, pure tone audiometry, or tympanometry. In that case, the ear complaints might be explained as a referred feeling from the irritated pharyngeal wall.

An interesting subgroup within the CRFP patients are the 4 patients (patients 7, 8, 9, and 13 on Table 2) who had no GER according to the criteria we used, but who nevertheless were included on the basis of their complaints and the signs found on clinical examination. These patients also responded favorably to their treatment. One possible explanation might be a placebo response. A second explanation, and the most plausible, is related to the established norms of 24-hour pH monitoring, especially regarding proximal acid exposure. The larynx and pharynx are not protected by the clearing mechanisms and intrinsic mucosal properties present in the distal esophagus. It is conceivable that a single episode of acid reflux may be sufficient to cause laryngeal (and most likely also pharyngeal) symptoms and signs. Delahunty and Cherry,⁸ who produced vocal fold granulomas experimentally in 1968, did so within 4 weeks by placing gastric juice on the vocal processes of dogs for only 30 min/d, 5 d/wk, with no exposure over the weekends. They produced erythema within the first week that did not resolve over the weekend at the end of the second week, and granulomas thereafter.⁸ If one were to accept a zero-exposure threshold for

proximal acid reflux, 2 of the 4 patients had evidence of proximal acid reflux (patients 7 and 8 on Table 2). Also, the normal pH monitoring results could reflect a day-to-day variability and the possibility of a false-negative finding on this basis. Therefore, these 4 patients may have responded favorably because they had (intermittent) reflux-induced laryngopharyngeal symptoms and signs even though they did not meet current diagnostic criteria on the day they were studied. On the other hand, this response could reflect a day-to-day variability of the 24-hour pH measurement and thus indicate a false-negative finding. If CSOM or CRFP persists in spite of antireflux therapy, additional (antibacterial) therapy might be prescribed or repeated pH monitoring might be performed to exclude ongoing acid reflux.

CONCLUSIONS

1. This is to date the largest and, to our knowledge, also the first study of groups of adult patients with reflux-related CSOM and with reflux-related CRFP. It documents the diagnostic workup and the results of antireflux therapy with omeprazole.

2. Ambulatory 24-hour dual-probe esophageal pH monitoring and upper gastrointestinal tract endoscopy are considered highly useful (certainly in CSOM patients) in establishing a GER diagnosis and in indicating the severity of GER. Additional information may be obtained by esophageal manometry.

3. The results of our reflux investigations indicate a higher prevalence of GER and usually more serious forms of GER in patients with CSOM than in patients with CRFP.

4. Antireflux treatment combining omeprazole and conservative antireflux measures appears to be highly successful in GER-related CSOM and CRFP.

5. The longer duration of antireflux therapy needed for CSOM patients to become free of symptoms and the higher maintenance dose required for them to remain symptom-free are also indicative of more serious forms of GER in CSOM patients than in CRFP patients.

6. In reflux-related ear problems, GER should be thought of as EER that may cause nasopharyngitis leading to eustachian tube dysfunction and/or middle ear disease.

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HEARING LOSS IN FINNISH ADULTS WITH OSTEOGENESIS IMPERFECTA: A NATIONWIDE SURVEY

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Hearing loss, bone fragility, and blue sclerae are the principal clinical features in osteogenesis imperfecta (OI), a genetic disorder of connective tissue. In a nationwide search, an audiometric evaluation of 133 adult patients was performed. According to the criteria introduced by Sillence, type I was the most common form of OI. Of the patients with normal hearing on audiometry, 17.1% reported subjective hearing loss, and 19.1% of the patients with impaired hearing did not recognize it. On audiometry, 57.9% of the patients had hearing loss, which was progressive, often of mixed type, and mostly bilateral, and began in the second to fourth decades of life. The frequency or severity of the hearing loss was not correlated with any other clinical features of OI. Hearing loss is common, affecting patients with all types of OI. Subjective misjudgment of hearing ability supports the need for repeated audiometry in all OI patients. A baseline study at the age of 10 years followed by audiograms every third year thereafter is recommended.

KEY WORDS — hearing loss, osteogenesis imperfecta.

INTRODUCTION

Progressive hearing loss is a common additional social and health problem in osteogenesis imperfecta (OI), a genetic disease of the skeleton. The principal clinical feature in OI is remarkable bone fragility that makes the bone susceptible to mild traumas. The propensity for fractures varies from a few fractures during the lifetime to hundreds in childhood. Many patients present with secondary deformities in the tubular bones, spine, and skull and with short stature, which together result in a remarkable physical and secondary psychosocial handicap from childhood onward. Dental discoloration and fragility (dentinogenesis imperfecta; DI) are also common.¹ The prevalence of OI is 1 in 10,000 persons to 1 in 30,000 persons.¹⁻³ The most common mode of inheritance is autosomal dominant, with large families with several affected generations and multiple affected members. Sporadic cases due to new dominant mutations are common. In addition, there are rare autosomal recessive forms of OI.⁴ Osteogenesis imperfecta is mainly caused by mutations in 1 of the 2 genes, COL1A1 and COL1A2, that encode the synthesis of type 1 collagen.¹

Although the oldest reports of OI date from the 17th century, and the oldest known case that was later diagnosed as OI is in a mummy dating from about

1000 BC, the classic symptoms of OI with bone fragility, blue sclerae, and hearing loss were first described by Adair-Dighton in a 1912 case report.⁵⁻¹⁰ The triad of OI, however, was first confirmed by van der Hoeve and de Kleyn in 1918, and was supported by several reports during the following years.¹¹⁻¹⁴

Since the late 1970s, OI has been divided into 4 major types on the basis of clinical, radiographic, and genetic criteria (Table 1^{4,15,16}). This classification, introduced by Sillence and later modified on several occasions, has replaced the earlier classification into congenita and tarda forms.^{4,15-17} The wide variation in expressivity and heterogeneity still today cause difficulties in classifying both familial and sporadic cases.^{18,19}

Six population studies on hearing loss in OI have been published.^{3,9,19-22} In these studies, hearing loss was found in 22.6% to 58% of OI patients.^{3,9,18,20-22} The hearing loss is progressive, often conductive at onset and later mixed or sensorineural, and it usually begins in the second or third decade of life.^{3,23-27} Only 4 previous studies have focused on hearing loss in different types of OI: Sillence et al¹⁸ and Paterson et al²² included all types of OI, and Garretsen et al²⁷ and Garretsen and Cremers²⁸ included OI type I only. Hearing loss appears to be most common in OI type I, affecting 35% to 78% of patients, and it has been

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TABLE 1. CLASSIFICATION OF OI

OI Type*	Clinical Features	Inheritance
IA	Mild to severe bone fragility, normal or slightly short stature, intensely blue sclerae, presenile hearing loss, normal teeth	AD
IB	Mild to severe bone fragility, normal or slightly short stature, intensely blue sclerae, presenile hearing loss, dentinogenesis imperfecta	AD
II	Extremely severe bone fragility	AD or AR
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity, short stature, bluish sclerae, variable dentin abnormality	AD or AR
IVA	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth	AD
IVB	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, dentinogenesis	AD
V	Hyperplastic callus OI type	AD
	Other types	
	Cole-Carpenter OI type	AD
	Bruck OI type	AR
	North American OI	AR
	Osteoporosis-pseudoglioma syndrome	AR

OI — osteogenesis imperfecta, AD — autosomal dominant, AR — autosomal recessive.

*Sillence classification, modified at Seventh International Conference on Osteogenesis Imperfecta, Montreal, 1999.^{4,15,16}

thought to be less common in OI type IV.^{4,19,22,27,28} In OI type III, Sillence⁴ found that only 1 of 6 patients had hearing loss, whereas Paterson et al²² reported that 52% of 207 patients had hearing loss. The definition of hearing loss, however, has varied greatly in the studies referred to, and the study subjects may have been selected.^{3,22-28}

The aim of this study was to evaluate the frequency and type of hearing loss in adult Finnish patients with a classified type of OI. We have previously reported on the hearing loss in OI children.²⁹

MATERIALS AND METHODS

Altogether, 254 OI patients were ascertained in a nationwide search through the patient register of the Department of Clinical Genetics, Helsinki University Central Hospital, a major referral center for genetic disorders of the skeleton and connective tissue in Finland, and through the membership register of the Finnish Osteogenesis Imperfecta Association, as well as the HILMO care register from Finnish university and local hospitals. The diagnosis was based on fracture history, clinical and radiographic findings, and family history. The number of patients ascertained corresponds to a prevalence of 4.9 per 100,000 persons.

The inclusion criteria for the present audiological study were OI, a previous audiometric study, and age over 16 years. The diagnosis was ascertained in all sporadic cases and in most familial cases by clinical and genetic evaluation at the genetics clinic of Helsinki University Central Hospital, and in the remaining cases through the hospital records. The patients

were approached through a questionnaire concerning the history of fractures, the color of the sclerae, DI, and hearing loss. They were asked about the subjective and objective onset of the hearing loss, the clinical course of the hearing deficit, the use of hearing devices, and surgical treatment. Clinical audiometry, including pure tone audiometry, speech audiometry, tympanometry, and measurement of the stapedial reflex, was performed under standard conditions in a soundproof room at a local secondary or tertiary care university hospital. The patients with no audiometric evaluation were excluded from the study, as were patients with non-OI-related ear disease. The questionnaire was returned, indicating consent to participate, by a total of 204 patients (80.3%), of whom 144 were adults. This report focuses on audiometric evaluation of the 133 adult patients, 17 years of age or older, who fulfilled the inclusion criteria. The mean age of the 50 men and 83 women was 40.8 years (SD, 15.98 years; range, 17 to 81 years). Twenty-nine of these patients (21.8%) had undergone a middle ear operation because of hearing loss caused by OI; altogether, 22 right ears and 19 left ears were operated on. A clinical examination of the patients with impaired hearing was performed to exclude causes other than OI for the hearing loss. For 131 patients, the type of OI could be determined by a clinical geneticist using the criteria introduced by Sillence.⁴ Subclassification of OI types I and IV into subtypes A and B (Table 1) on the basis of presence or absence of DI was not used in our audiological study, since the dental manifestations of OI apparently form a continuum from normal dentin to severe DI, and visual evaluation does not reveal the changes seen on radiography or light and transmission electron microscopy.³⁰

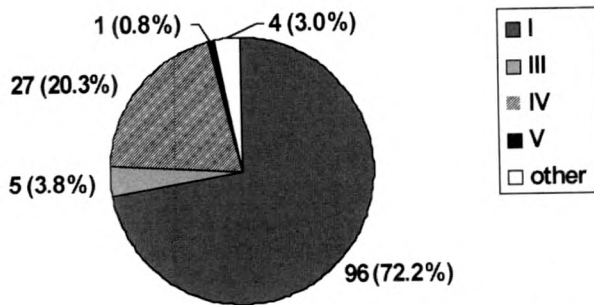


Fig 1. Classification of osteogenesis imperfecta (OI) in 133 Finnish adults.

Normal hearing was defined as a pure tone average at 0.5, 1, and 2 kHz that was equal to or better than 15 dB hearing level (HL) in patients under 60 years of age, and equal to or better than 20 dB HL in patients of 60 years or older. Definitions for different types of hearing loss paralleling the definitions given by Shapiro et al³¹ and Pedersen³ were used, as follows.

1. Conductive hearing loss: an average air-bone gap for the frequencies 0.5, 1, and 2 kHz of greater than 15 dB with a corresponding bone conduction threshold of better than 15 dB.
2. Sensorineural hearing loss: air conduction thresholds for the frequencies 0.5, 1, and 2 kHz of equal to or greater than 15 dB with a corresponding air-bone gap smaller than 15 dB.
3. Mixed hearing loss: an average air-bone gap for the frequencies 0.5, 1, and 2 kHz of greater than 15 dB with a corresponding bone conduction threshold equal to or greater than 15 dB.

RESULTS

Of the total of 133 adult patients in whom audiometry had been performed, 111 patients (83.4%) reported blue sclerae, and all except 1 had had fractures. Fifty-two patients (39.4%) reported DI, and 83 (62.4%) had the familial type of OI. Twenty-eight patients (21.1%) had continuous tinnitus, and 61 (45.9%) had intermittent tinnitus. Fifty-seven patients (43.8%) reported recurrent or continuous vertigo, which could be classified as inner ear-related by case history in 28 patients (21% rotational or swaying ver-

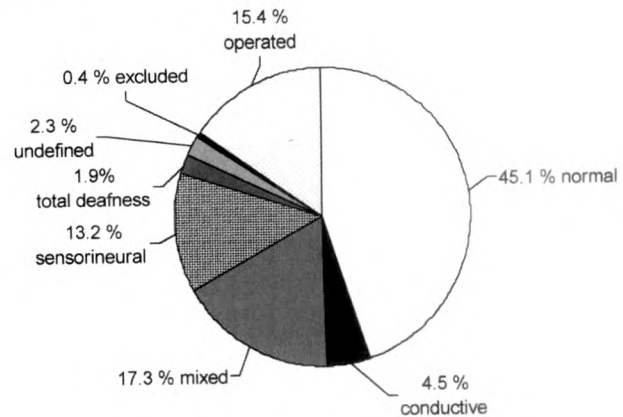


Fig 2. Hearing pattern in 266 OI ears.

tigo). Thirty-three patients (24.8%) used a hearing aid. All 133 cases except 2 could be classified according to Sillence⁴ (Fig 1). Type I was the most common form of OI. The sex and age distribution of the patients is presented in Table 2.

The results of audiometric evaluation of 266 OI ears are presented in Fig 2. The subjective sensation of hearing ability was compared with the audiometric findings in the 133 patients studied. Of the patients with normal hearing on audiometry, 17.1% reported hearing loss, and on the other hand, 19.1% of the patients with hearing loss were not aware of any hearing loss. At audiometry, 63 patients (47.4%) had bilateral hearing loss, and 77 (57.9%) had hearing loss in at least one ear (mean age, 45.4 years; range, 17 to 81 years; SD, 16.4 years). In addition, 1 patient had accidental unilateral total deafness caused by a basal skull fracture. The mixed type of hearing loss was most common: 17.3% of the ears. The type of hearing loss could not be defined for 6 ears. In addition, 22 right and 19 left ears of, altogether, 29 patients had been operated on because of conductive or mixed hearing loss caused by OI, with bilateral stapedotomy in 12 of the cases. The hearing loss increased with age. The mean age at the onset of hearing loss was, according to patient history or audiometry, 29.0 years (range, 7 to 81 years; SD, 14.8 years; Fig 3). Both mixed and sensorineural hearing losses occurred in all age groups, while conductive hearing loss was predominantly seen in younger patients (Fig

TABLE 2. CLASSIFICATION OF PATIENTS BY SEX, AGE, AND OI TYPE

	I	IA	IB	III	IVA	IVB	III/IV	V	Unclassifiable	Total
Male	5	23	5	3	5	6	1	1	1	50
Female	5	50	8	2	5	11	1	0	1	83
Total	10	73	13	5	10	17	2	1	2	133
Mean age (y)	52.3	41.3	46.0	36.2	35.2	35.0	42.5	43.0	31.0	40.8
Range (y)	33-81	17-87	19-69	25-47	17-51	20-65	28-57		24-38	17-81
SD (y)	16.5	17.3	16.2	9.4	10.8	14.2				16.0

Types I and IV were divided into A and B by visual evaluation of teeth. Ten patients could not be reliably placed in A or B group of type I.

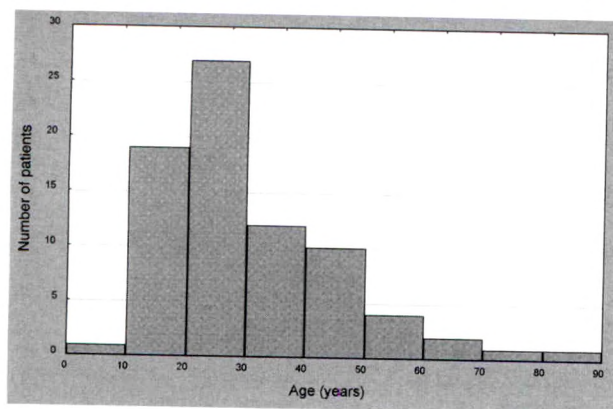


Fig 3. Age at onset of hearing loss in 77 patients. Youngest patient was 7 years old. Twenty-six percent of patients had symptomatic hearing loss before age of 20 years, 61% before age of 30 years, 76.6% before age of 40 years, and 89.6% before age of 50 years.

4). Hearing loss above 30 dB HL in both ears was found in 40 of the 133 patients (30.1%; Fig 5).

The frequency of hearing loss in classified types of OI is presented in Fig 6. The hearing pattern in different types of OI is presented in Fig 7, and the severity of hearing loss in classified types of OI, with operated patients excluded, is presented in Fig 8. No correlation was found between the frequency or severity of hearing loss and the type of OI. The types of hearing loss for the right ear were significantly different between OI types I and IV ($p = .028$), indicating that inner ear-related hearing loss is more common in OI type I. Although a significant difference was not found for the left ear, the tendency was similar ($p = .09$).

A strong correlation was found between the hearing levels of the right and left ears of individual patients, indicating that hearing loss eventually affects both ears (Pearson correlation coefficient, 0.71). The type of hearing loss was usually the same for both ears. The type of hearing loss, especially the mixed type, was similar within the families studied, but no statistical significance could be shown because of

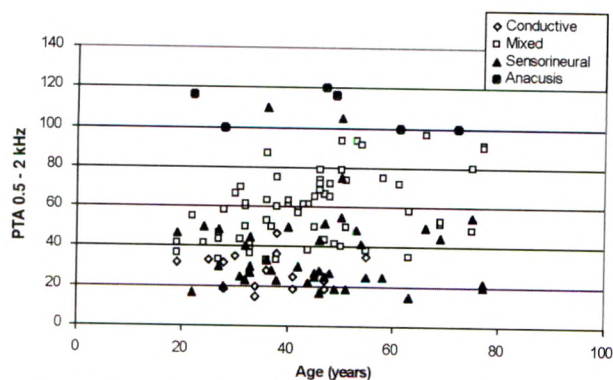


Fig 4. Type of hearing loss related to age. PTA_{0.5-2kHz} — pure tone average at 0.5, 1, and 2 kHz.

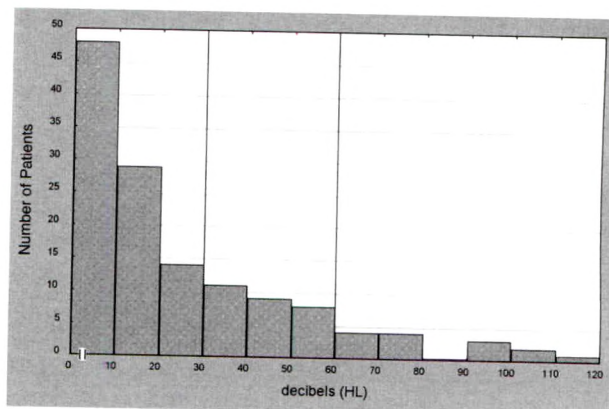


Fig 5. PTA_{0.5-2kHz} of better ear in 133 OI patients. Hearing loss was socially disturbing in 30.1% of patients (40/133).

the small family size: only 1 family had as many as 6 members with OI, and most of the families included 3 or fewer OI patients. No correlation was found between occurrence of hearing loss and blue sclerae, number of fractures, DI, vertigo, or whether the OI was sporadic or familial; nor between the different types of hearing loss and DI, blue sclerae, vertigo, number of fractures, or whether the OI was sporadic or familial. Tinnitus was most frequent in patients with sensorineural or mixed hearing loss, and the frequency of tinnitus increased with age.

Three patients were found to have total deafness not related to surgery. A woman with mild OI type I developed total deafness at the age of 71 years after progressive, OI-related hearing loss. In addition, a 47-year-old man with blue sclerae and DI, but no fractures, had total deafness of the right ear. The objective onset of hearing loss remained unclear, but subjective hearing loss was reported at the age of 7 years. However, in this patient, audiometry was first performed at the age of 20 years. Another woman, 49 years of age, had rapidly progressive hearing loss that resulted in total deafness in early adulthood. She had nonfamilial OI type I. The sclerae were blue, the teeth were normal by visual evaluation, and she had had multiple fractures. Since the age of 15 years, she

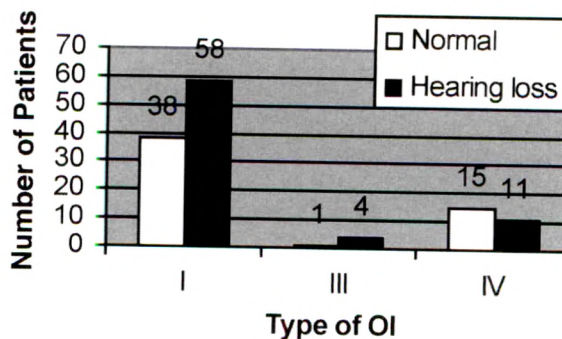


Fig 6. Frequency of hearing loss in classified types of OI (N = 131, including operated patients).

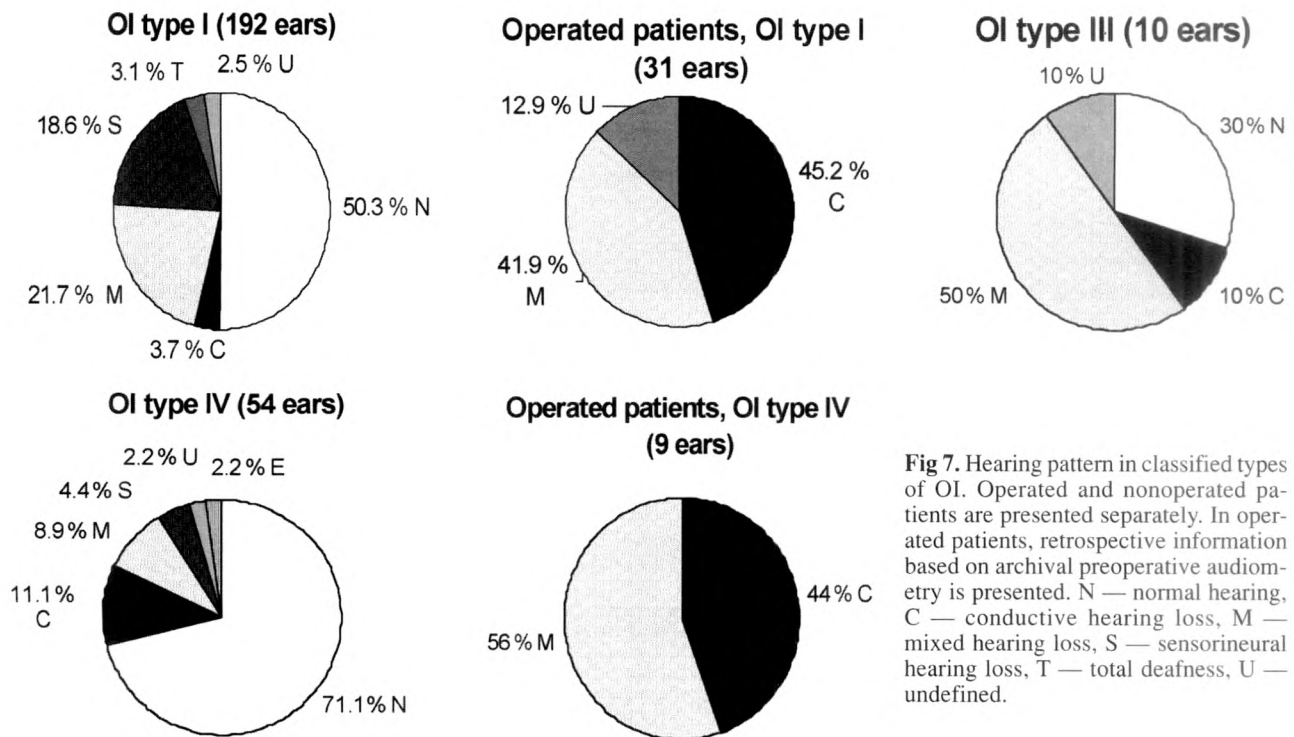


Fig 7. Hearing pattern in classified types of OI. Operated and nonoperated patients are presented separately. In operated patients, retrospective information based on archival preoperative audiometry is presented. N — normal hearing, C — conductive hearing loss, M — mixed hearing loss, S — sensorineural hearing loss, T — total deafness, U — undefined.

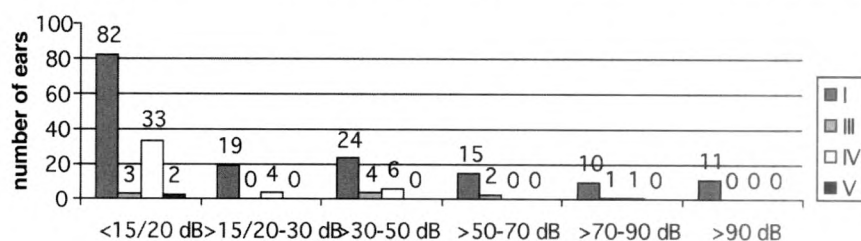
had had slowly progressive hearing loss that, at the time of the first audiometry at the age of 20 years, was of mixed type. The hearing loss then progressed rapidly, resulting in total deafness at the age of 22 years (Fig 9). In addition, 8 nonoperated patients were found to have sensorineural hearing loss before the age of 40 years. Seven of these patients had OI type I, and 1 patient with blue sclerae and DI was not classified. The mean age of these patients was 26 years at the time of the study (SD, 26.0 years; range, 17 to 37 years), and the mean age at the onset of the hearing loss was 22.1 years (SD, 7.0 years; range, 15 to 36 years). The mean pure tone average at 0.5, 1, and 2 kHz was 39.2 dB HL in the right ear and 32.9 dB HL in the left ear (SD, 13.4 dB HL in the right ear and 17.1 dB HL in the left ear; range, 20 to 55 dB HL in the right ear and 15 to 66.7 dB HL in the left ear).

DISCUSSION

This study is a population-based survey on a national level. We examined hearing loss in OI patients classified according to Sillence⁴ and performed audiometric evaluation in all patients. The low preva-

lence of OI in Finland found in our study, 4.9 per 100,000 persons, may be explained by the variable severity of clinical expression, nonreporting, and lack of patient registers, as in former studies on OI.¹ It is a general impression that about half of OI patients have progressive hearing loss (Table 3^{3,21,22,25-28}). In most studies, the hearing loss is found to be bilateral and progressive. Hearing loss starts in the second or third decade of life, and it is often conductive at the onset and later mixed or sensorineural. However, the definition of hearing loss has varied greatly, and the study subjects may have been selected in previous studies.^{3,22-28} The first population study on OI, by Seedorff⁹ in Denmark in 1949, showed a 28% incidence of hearing loss, and Smår²⁰ in Sweden in 1961 found a 22.6% incidence of hearing loss. More recently, Pedersen³ in Denmark found a 50% incidence of hearing loss among 173 patients with OI classified into congenita and tarda forms. The hearing loss was predominantly of mixed type and started in the second or third decade of life. The median age at onset was 25 years. In the 1989 study of Stewart and O'Reilly,²¹ 46% of the Scottish OI patients had hearing loss, progressing from the conductive type in the

Fig 8. Severity of hearing loss in OI types I, III, IV, and V. In addition to 41 operated ears not presented in this Figure, significant hearing loss was observed in 104 of 224 ears studied.



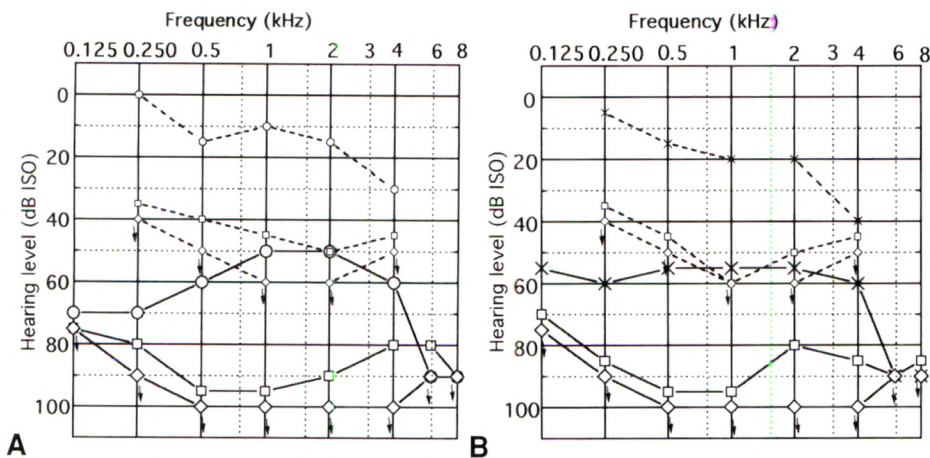


Fig 9. Conductive hearing loss resulting in total deafness at age of 22 years. Pure tone audiometry of **A)** left and **B)** right ears at age of 20 years (crosses), at age of 22 years (squares), and at age of 28 years (diamonds). Solid lines — air conduction threshold; dotted lines — bone conduction threshold. ISO — International Standards Organization.

second or third decade of life to a mixed type later in life. In a 2001 population study by Paterson et al,²² subjective hearing loss was reported by 22.7% of the British OI patients, starting during the first 4 decades of life. Also in other, non-population studies, the hearing loss has been seen to start in early adulthood, proceeding from conductive hearing loss to a mixed and sensorineural type with increasing age.^{24,25} Sensorineural hearing loss beginning as a mild high-frequency hearing loss and expanding to involve the lower frequencies with time has also been reported.³¹ In some studies, a mixed hearing loss has been found to be the most frequent, with conductive hearing loss only in some younger patients, and sensorineural hearing loss and deafness in constant proportions independent of age.^{27,28}

In our study, hearing loss was found to be progressive and bilateral, beginning predominantly in the second to fourth decades of life, with a mean age of 29.0 years at the onset of the hearing loss. Hearing loss, however, may begin in childhood, as indicated in earlier studies.^{3,17,21,27-29} Of the 133 patients we studied, 57.9% ($n = 77$) had a hearing loss, which was bilateral in 47.4% of them ($n = 63$). This finding

supports the general impression that 50% of OI patients have a hearing impairment.^{3,9,18,20,21} Mixed hearing loss was the most frequent type of hearing loss, occurring in 17.3% of the 266 ears. In addition, the preoperative hearing loss was of mixed type in 19 of the 41 operated ears (46.3%). The small amount of conductive hearing loss in this series, only 4.5% of the ears, is explained by previous stapedotomy; 18 of these operations were performed for conductive hearing loss (43.9%). Conductive hearing loss was mostly observed in the younger patients, while sensorineural and mixed types of hearing loss were seen in all age groups, as reported in earlier studies.^{3,23-27}

No correlation was found between the hearing loss and other OI features such as blue sclerae, frequency of fractures, and DI. Even though no correlation analysis could be done between hearing loss and familiarity of OI because of the small size of the families, the earlier indication of deafness as a familial feature is supported by our study.²²

In the few previous studies using the present classification of OI, hearing loss is suggested to be more

TABLE 3. HEARING LOSS IN ADULTS WITH OI

Authors	Year	No. of Patients	Selected Patients	Definition of Hearing Loss*	Audiometry	Hearing Loss	
						No.	%
Riedner et al ²⁵	1980	70	13 Families	Yes	Yes + portable	26/52	50.0
Cox and Simmons ²⁶	1982	30	5 Families	Yes	Portable	6/12	50.0
Pedersen ³	1984	173	Population	Yes	Yes + portable	73/114	64.0
Stewart and O'Reilly ²¹	1989	56	Population	Yes	Yes + portable	29/40	72.5
Garretsen and Cremers ²⁸	1991	70	OI type I	Yes	Yes	15/31	48.4
Garretsen et al ²⁷	1997	142	OI type I, selected patients	Yes	Yes	83/91	91.2
Paterson et al ²²	2001	1,394	Population	No	No	297/928	32.0
This study		133	Population	Yes	Yes	77/133	57.9

In previous studies, adult patients have been defined as patients ≥ 20 years of age, with exception of study of Paterson et al,²² in which patients were ≥ 10 years of age. In present study, adults are defined as patients ≥ 17 years of age. Percentage figures are influenced by age structure of population in question, which is not reported in these studies.

Portable — portable audiometry, in addition to clinic audiometry.

*Definition varies among different studies.

common in OI type I than in OI type IV. In the 1979 Sillence et al¹⁸ study from Victoria, Australia, hearing loss was found in 35% of OI type I patients of all ages. No hearing loss was found in OI type IV; there were, however, only 8 patients with OI type IV in the study material. In the 1983 study of Paterson et al,³² subjective hearing loss was reported by 50% of type I and by 29.4% of type IV patients over 30 years of age, but there was no significant difference (69 versus 48 patients). In a later study by Paterson et al,²² about 30% of type IA, IB, and IVB patients reported hearing loss at the age of 30 years, while the proportion was significantly lower in OI type IVA (9%). Garretsen and Cremers²⁸ found that 43% of 70 patients with OI type I had hearing loss. In a later study by Garretsen et al,²⁷ hearing loss was found in 79% of type I OI patients. The high rate of hearing loss is explained by selection of the patients, who either had been scheduled for ear surgery or volunteered for the study because of hearing loss. Only 2 studies focusing on hearing loss in OI type III have been published, with different outcomes: 52% with hearing loss at the age of 30 years in a study by Paterson et al²² on 206 type III OI patients, and only 1 of 6 type III OI patients with hearing loss in a study by Sillence et al.¹⁸ In the former study, hearing loss was based on the subjective experience of the patients (personal communication), and in the latter, no definition for hearing loss was given.

In our study, no correlation could be found between the types of OI and the frequency or severity of the hearing loss: 60.4% of the type I OI patients and 42.3% of the type IV OI patients had hearing loss. The types of hearing loss, however, seemed to be slightly different between types I and IV: inner ear-related hearing loss occurred more often in OI type I. Of the 5 type III OI patients, 4 had hearing loss,

supporting the finding by Paterson et al²² that hearing loss is common in OI type III.

Although the hearing loss in OI is clinically otosclerosis-like, OI and otosclerosis are two separate diseases with clinical similarities.^{3,33-37} Compared with otosclerosis, the hearing loss in OI has a tendency toward earlier onset, more severe middle ear involvement, and a higher incidence of sensorineural hearing loss.^{21,23,32,35} In our study, sensorineural hearing loss was observed also in the youngest age groups, indicating in OI a group of patients with a predominantly sensorineural pattern at the onset of hearing loss, which is rare in otosclerosis. Early sensorineural hearing loss was found only in patients with OI type I, and the only patient with rapidly progressing hearing loss, resulting in total deafness within 8 years at the age of 22 years, also had OI type I. In earlier studies, total deafness is reported mostly in older age groups, but sensorineural hearing loss at an early age is also reported.^{3,21,29} The cause of early sensorineural hearing loss in OI remains unresolved, and to date, it has not been possible to establish genotype-phenotype correlations.

Hearing loss is a common feature in OI, affecting patients with all types of OI. The subjective assessment of hearing ability may be misleading: almost 40% of the patients in this study misjudged their hearing ability. This finding supports the need for repeated audiometry in all patients with OI. Because early detection and treatment of hearing loss is of utmost importance to avoid aggravation of the physical handicap, audiometry should be performed in all OI patients as a baseline study at the age of 10 years, with repeated audiograms every third year thereafter, even for asymptomatic patients. Further studies based on molecular genetic classification of OI are needed to reveal the pathogenesis of hearing loss.

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INDUCTION OF GROWTH FACTOR EXPRESSION IS REDUCED DURING HEALING OF TYMPANIC MEMBRANE PERFORATIONS IN GLUCOCORTICOID-TREATED RATS

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The participation of growth factors in wound healing of tympanic membranes (TMs) is established. To determine the possible role of these growth factors in normal healing, we examined the regulation of keratinocyte growth factor (KGF), transforming growth factor- α (TGF- α), and basic fibroblast growth factor (bFGF) messenger RNA (mRNA) expression in wounded TMs of glucocorticoid-treated rats; these rats have severe wound healing abnormalities. Induction of KGF, TGF- α , and bFGF mRNA expression after TM injury was significantly reduced in these rats. Moreover, we found that the average number of bromodeoxyuridine-positive cells in a glucocorticoid-treated group was significantly lower than that in controls. The data suggest that reduced expression of these genes might be partially responsible for the wound healing defects seen in these animals. These results provide a possible explanation for the beneficial effect of exogenous KGF, TGF- α , or bFGF in treatment of wound healing disorders of the TM.

KEY WORDS — glucocorticoid, growth factor, tympanic membrane, wound healing.

INTRODUCTION

The regeneration of the tympanic membrane (TM) after a traumatic perforation is a complex biological process that involves epithelial proliferation, epithelial migration, fibroblast proliferation, angiogenesis, and tissue remodeling.¹⁻³ Previous studies indicate that a variety of growth factors and cytokines are key modulators of this process. For example, local application of epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) accelerates the rate of closure of TM perforations.⁴⁻⁶ In addition, immunohistochemical reactivity for endogenous bFGF, EGF, and transforming growth factor- α (TGF- α) is induced during TM perforation healing.^{7,8} We previously demonstrated that keratinocyte growth factor (KGF) and TGF- α messenger RNA (mRNA) expressions were rapidly induced in the early stage of TM repair, while the expression level of bFGF was gradually induced in the later stage of this process.⁹

To determine whether the induction of these growth factors is important for normal repair, we developed a model of impaired wound healing of the TM in glucocorticoid-treated rats. The regulation of KGF, TGF- α , and bFGF mRNA expression and the number of bromodeoxyuridine (BrdU)-labeled cells in wounded TMs of these animals were examined, and the suppressive effects of glucocorticoids on mRNA expression of KGF, TGF- α , bFGF, and DNA synthesis in wounded TMs were demonstrated. The data suggest that reduced expression of these genes might

be at least partially responsible for the wound healing defects seen in these animals.

MATERIALS AND METHODS

Glucocorticoid Treatment of Rats. Adult male Wistar rats (weight, 230 to 300 g) were used in this study. All experiments were performed according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. For each experiment, 7 rats were given subcutaneous injections at 9:00 AM daily with dexamethasone (1 mg/kg body weight). Seven control rats were injected with phosphate-buffered saline solution (PBS). During the wound healing period, the daily injection of dexamethasone or PBS was continued.

Operative Procedure. Three independent wound healing experiments were performed. For each experiment, 7 dexamethasone-treated rats and 7 control rats were anesthetized with intraperitoneal injections of sodium pentobarbital (0.2 mL/100 g body weight). On the sixth day of treatment with either dexamethasone or PBS, 1.5-mm perforations were made in the posterior pars tensa of the TM in each ear with a 22-gauge needle under sterile conditions with an otomicroscope. Animals that demonstrated signs of external otitis or otitis media before or after the surgery were excluded from the study.

Comparison of TM Perforation Closure Rates in Glucocorticoid-Treated and Control Rats. Five TMs

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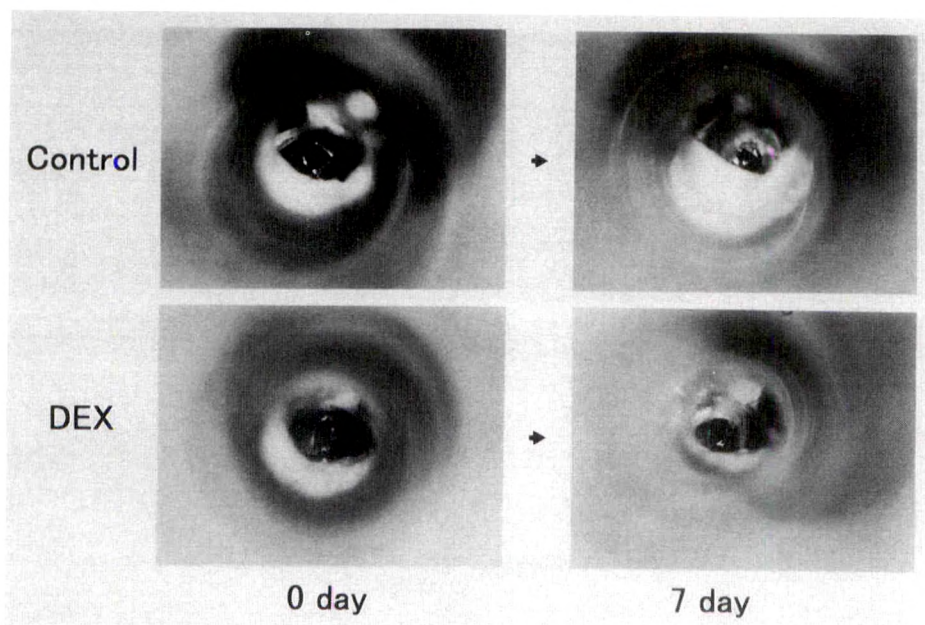


Fig 1. Photographs of tympanic membrane (TM) perforations in dexamethasone-treated (DEX) and control rats at 0 and 7 days after wounding.

from dexamethasone-treated rats and 5 TMs from control rats were photographed with an operating otomicroscope (Karl Zeiss, Berlin, Germany) 0 and 7 days after wounding with anesthesia. Each photograph was digitally scanned into a Power Macintosh computer with an image scanner. The perforation size and the upper posterior area of the TM for each photograph were calculated with NIH Image 1.62 software (Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland). The perforation size for each photograph was calculated by computer as a proportion of the upper posterior area of the TM as described previously.¹⁰ In this way, interphotograph variation in magnifica-

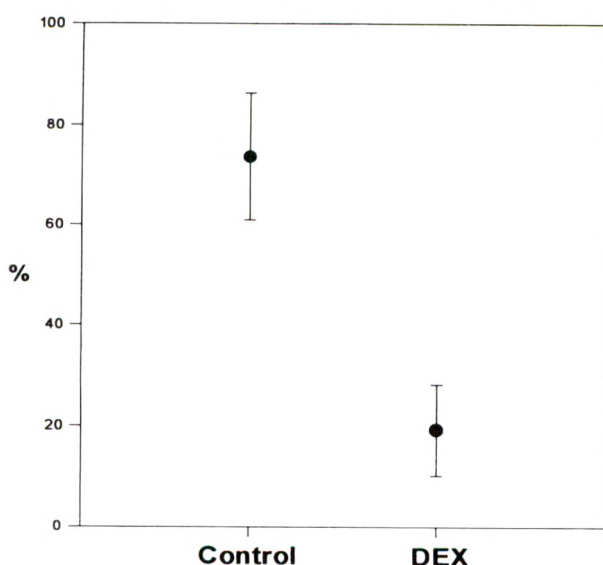


Fig 2. Comparison of closure rates of TM perforation between DEX rats and controls, 7 days after wounding. Average closure rate was higher in control group than in DEX group.

tion of the TMs and perforations was factored out. The percentage of TM closure was determined by comparing the compensated areas of TM perforation on day 7 with those on the day of wounding.

Histologic Analysis. Tympanic membrane perforations were created as described above. Six dexamethasone-treated rats and 6 control rats were injected with BrdU (Sigma, St Louis, Missouri; 60 mg/kg body weight) intraperitoneally on the seventh day after wounding. Two hours after injection, the animals were deeply anesthetized by intraperitoneal injections of pentobarbital and decapitated. The temporal bones were removed and fixed in 10% formalin for 2 weeks. After fixation, the specimens were decalcified in 10% ethylenediaminetetraacetic solution for 10 days and then embedded in paraffin. The temporal bones were cut into 5- μ m sections continuously in a plane horizontal to the malleus handle, from above the short process of the malleus to below the umbo. The tissue sections from every 50 μ m were mounted on glass slides. Some slides were stained with hematoxylin and eosin. Other slides were used for immunohistochemical analysis using monoclonal anti-BrdU antibody (Dako, Copenhagen, Denmark) to examine the mitotic cells in the regenerating TMs.

BrdU Immunohistochemical Staining. Some of the serial sections were deparaffinized with xylene, dehydrated with a descending ethanol series, washed in PBS, and digested with 0.25% pepsin/0.1N hydrochloric acid solution (Kreateck, Amsterdam, the Netherlands). After a rinsing in PBS, the endogenous peroxidase was quenched with 3% hydrogen peroxide for 5 minutes. The slides were blocked with a 10% horse serum (Vector, Burlingame, California)

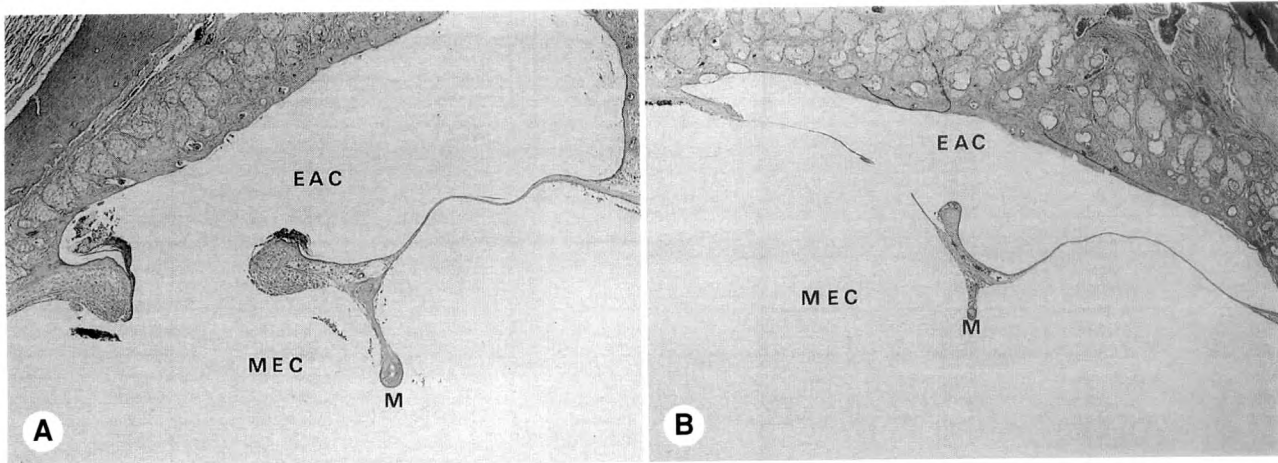


Fig 3. Histologic findings of TM perforation in both DEX and control rats 7 days after wounding (H & E). EAC — external auditory canal, MEC — middle ear cavity, M — malleus. **A)** Both epidermal and middle layers were markedly thickened around edge of perforation in control rats. **B)** In contrast, neither epidermal nor fibrous reaction at edge of perforation was noted in DEX rats, but there was small amount of epidermal hyperplasia short distance from perforated edge.

for 20 minutes and then incubated with the anti-BrdU antibody (DAKO), diluted 1:50 in PBS, for 20 minutes at room temperature. After washes, sections were incubated with biotinylated anti-mouse IgG (Vector), diluted 1:200 for 30 minutes, and placed into peroxidase-linked avidin-biotin (Vector). The slides were then exposed to 3,3'-diaminobenzidine tetrahydrochloride (DAB; MBL, Nagoya, Japan) and counter-

stained with hematoxylin.

BrdU-Positive Cell Count. The sections were examined under a light microscope at a magnification of 100 $\times$ (Olympus, Tokyo, Japan). Three different sections containing TM perforations were chosen at random for each ear. Eighteen sections from each group were analyzed for BrdU-positive cell counts.

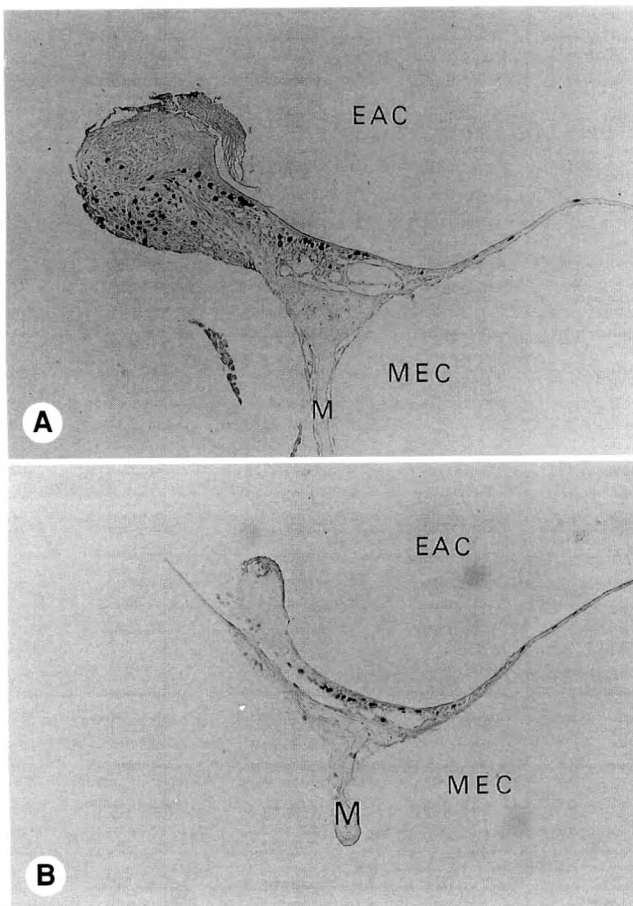
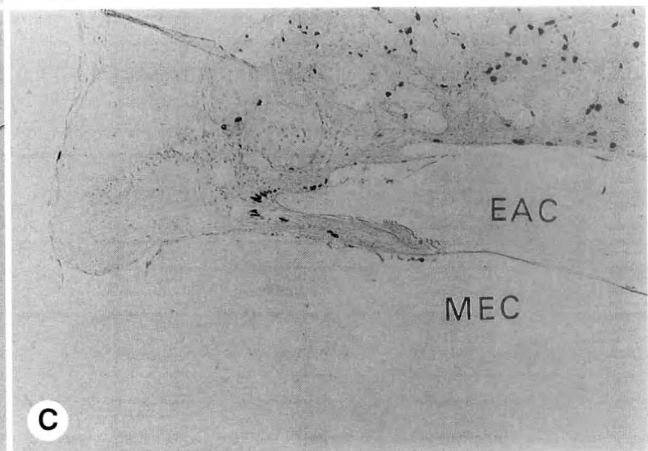


Fig 4. Bromodeoxyuridine (BrdU)-labeled cells in TMs of **A)** control rat and **B,C)** DEX rats on seventh day after wounding.



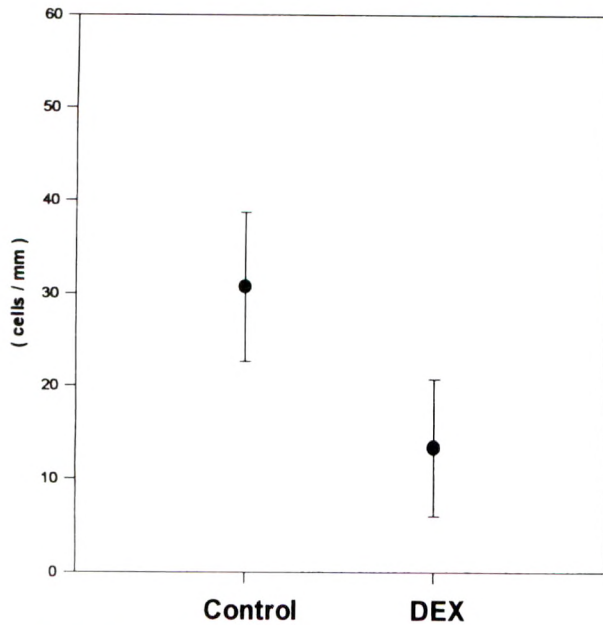


Fig 5. Comparison of numbers of BrdU-labeled cells in regenerating TMs between DEX rats and controls, 7 days after wounding. Values represent mean $\pm$ SD. By Student's *t*-test, $p < .0001$.

The number of BrdU-positive cells were counted from one perforation edge to the annulus and then from the other perforation edge to the other annulus. The length of the TM was also measured. The average number of labeled cells along 500 μ m of the TM in each group was compared to that of the control group. Student's *t*-test was used to assess the significance of differences between the two groups.

Preparation of Wounded Tissues for RNA Isolation. Four TMs from 2 dexamethasone-treated rats and 4 TMs from control rats were obtained on days 1, 3, and 5 after wounding. Unwounded TMs from dexamethasone-treated and control rats were collected for comparison. The pars tensa of the TMs with the malleus was removed quickly after making an incision along the annulus of the TM. The TMs were dissected out from the malleus, immediately frozen, and stored at -70°C for subsequent RNA isolation.

Semiquantitative RT-PCR. Total RNA was isolated from frozen TM samples according to the acid guanidinium-phenol-chloroform method.¹¹ One microgram of total RNA from each sample was subjected to reverse transcription-polymerase chain reaction (RT-PCR). The primers chosen for amplification of KGF, bFGF, TGF- α , and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were described previously.⁹ The PCR was performed as previously described, and PCR products were size-fractionated by electrophoresis through a 2.5% agarose gel, then visualized by ethidium bromide staining, and transferred onto nylon membranes according to the Southern meth-

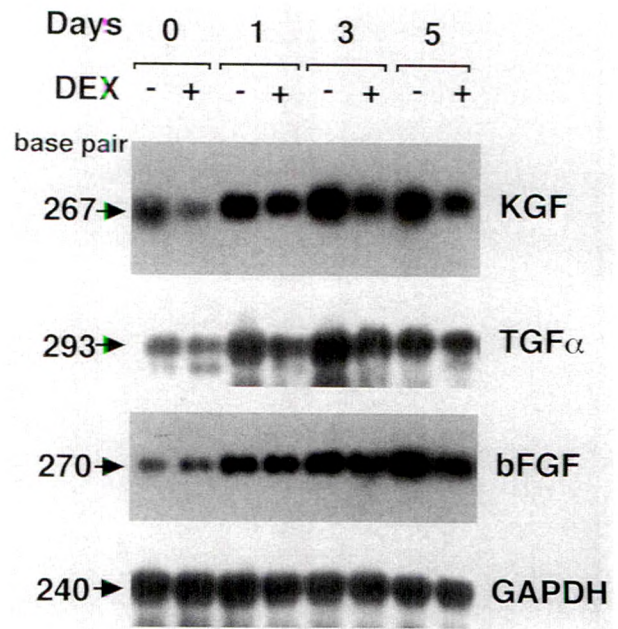


Fig 6. Southern blot of reverse transcription-polymerase chain reaction (RT-PCR) products from TM in glucocorticoid-treated and control rats at various intervals after wounding for keratinocyte growth factor (KGF), transforming growth factor- α (TGF- α), basic fibroblast growth factor (bFGF), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using specific oligonucleotide probes. Arrows indicate predicted size of RT-PCR products for each gene.

od.¹² The membranes were hybridized with nonradioactive oligo probes specific for a sequence internal to the primers used in the PCR amplification described previously.⁹ A digoxigenin oligonucleotide 3'-end labeling kit (Roche Diagnostics, Basel, Switzerland) was employed for nonradioactive hybridization of PCR products according to the manufacturer's instructions.

Semiquantitative Analysis of PCR Products. The exposed films were scanned into a Power Macintosh computer and quantification of transcript levels was performed with NIH Image 1.62 software. To adjust for potential differences in the actual amount of complementary DNA (cDNA) used in each PCR reaction, the amount of target cDNA was quantified relative to the GAPDH level of each sample and was expressed in relative units of each gene. The data are mean values from 3 independent experiments.

RESULTS

Photographs of TM Perforations in Glucocorticoid-Treated and Control Rats at 0 and 7 Days After Wounding. The TM perforations were observed and photographed with the aid of the otomicroscope at 0 and 7 days. At 7 days, the TMs in the control rats showed a scarce proliferative reaction with a thick-

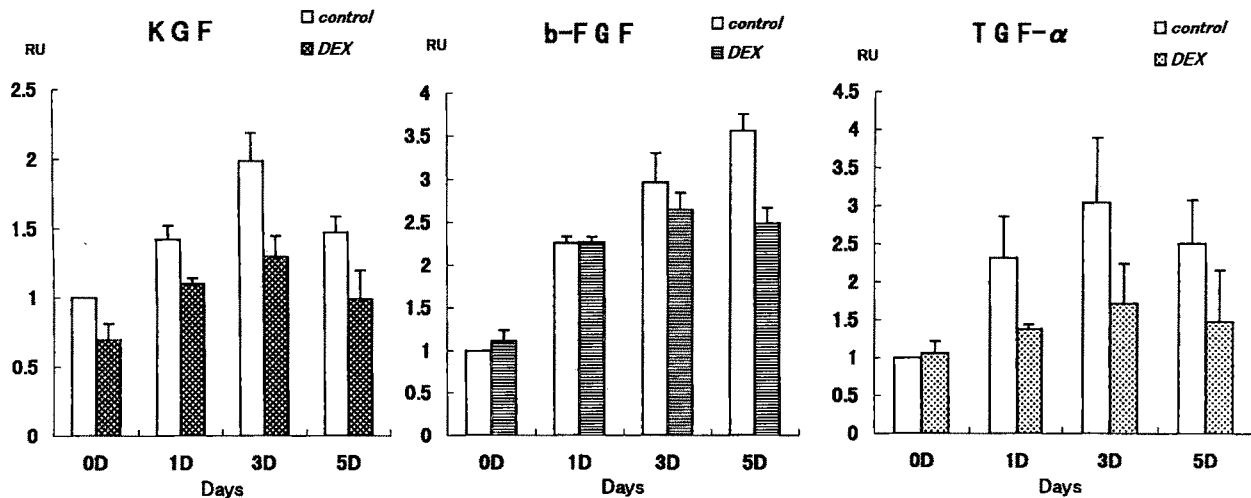


Fig 7. Quantitative comparative analysis of KGF, TGF- α , and bFGF bands as shown in Fig 6. To adjust for potential differences in actual amount of complementary DNA (cDNA) used in each PCR reaction, amount of target cDNA was quantified relative to GAPDH level of each sample. Values represent mean $\pm$ SD of 3 independent experiments. RU — relative units.

ened epithelial ridge around the partially closed perforation, while the TMs in the dexamethasone-treated rats remained thin, presenting no epithelial ridge proliferation around the perforation (Fig 1).

To compare the closure rates of TM perforations between the two groups, we measured the areas of the TM perforations at 0 and 7 days in 5 control rats and 5 dexamethasone-treated rats with NIH Image. The average closure rate was much higher in the control group than in the dexamethasone-treated group: $73.6\% \pm 12.7\%$ and $19.2\% \pm 9.0\%$, respectively (Fig 2). The difference was statistically significant at $p < .0001$ by Student's paired t -test. These results demonstrate that the closure of the TM perforations in the dexamethasone-treated rats was severely inhibited.

Histologic Section of TM Perforation of Glucocorticoid-Treated and Control Rats 7 Days After Wounding. Both the epidermal layer and the middle layer were markedly thickened around the edge of the perforation in every specimen from the control rats (Fig 3A). On the other hand, neither an epidermal nor a fibrous reaction at the edge of the perforation was noted in the dexamethasone-treated rats, but there was some epidermal hyperplasia a small distance away from the perforation edge in every specimen (Fig 3B). It appears that the glucocorticoid arrested the migrating epidermis at the perforation site, but not over the whole TM.

BrdU-Labeled Cell Analysis in TM in Glucocorticoid-Treated and Control Rats 7 Days After Wounding. Many BrdU-labeled cells were observed in the middle and epidermal layers of the TM in control rats on the 7th day after wounding (Fig 4A), but few BrdU-positive cells were observed at the outer lay-

ers of the TM around the malleus handle and posterior annulus in dexamethasone-treated rats (Fig 4B,C). The average numbers of BrdU-positive cells were counted along 500 μ m of the TM in 21 slides from each group. Figure 5 compares the numbers of BrdU-positive cells in the regenerating TM between the two groups. There were 2.3-fold fewer BrdU-positive cells in the dexamethasone-treated group than in the controls, a statistically significant difference ($p < .001$).

Semiquantitative Assessment of KGF, TGF- α , and bFGF Expression in Wounded TMs in Glucocorticoid-Treated and Control Rats. To compare the induction of growth factors in the wounded TMs of glucocorticoid-treated and control rats, semiquantitative RT-PCR was undertaken to examine the time course of KGF, TGF- α , bFGF, and GAPDH expression in rat TMs at 1, 3, and 5 days after wounding. A PCR was performed for 30 amplification cycles for KGF and TGF- α , 30 amplification cycles for bFGF, and 25 amplification cycles for GAPDH; these cycle numbers were found to lie optimally within the logarithmic amplification range by kinetic studies. As shown in Fig 6, the induction of each growth factor was decreased in dexamethasone-treated rats.

In the control rats, KGF and TGF- α mRNA expressions were induced 2.0-fold and 3.0-fold, respectively, peaking in 3 days and subsequently declining. In contrast, bFGF mRNA expression increased gradually and remained elevated for 5 days. In the dexamethasone-treated rats, KGF and TGF- α mRNAs were induced, peaking in 3 days. However, the amounts of induction were significantly lower (1.3-fold and 1.7-fold, respectively) than that in the control rats (Fig 7). The induction of bFGF mRNA in dexamethasone-treated rats was similar to that in con-

control rats during the first 3 days after injury, then subsequently declined (Fig 7). These results demonstrate that the decline of the expression levels of KGF, TGF- α , and bFGF might be partially responsible for the wound healing defect of the TMs seen in dexamethasone-treated rats.

DISCUSSION

Wound healing proceeds through a series of coordinated cellular- and cytokine-mediated events that result in the restoration of functional integrity of tissue.¹³ A number of conditions, such as immunodeficiency, venous stasis, diabetes mellitus, and the prolonged administration of anti-inflammatory steroids, result in delayed wound healing.^{14,15} In this study, we present a model of impaired wound healing of the TM in glucocorticoid-treated rats. The wound healing of the TM in these rats is markedly delayed compared to that of normal controls, and the healing impairment is histopathologically characterized by reduced formation of granulation tissue and cellular infiltration at the edge of the perforation.

Glucocorticoid is a potent anti-inflammatory agent that both stimulates and inhibits the transcription of a variety of genes.¹⁶ The prolonged administration of anti-inflammatory steroids leads to a delay in wound healing of the skin and an increase in local wound complications.¹⁷ Although topical application of hydrocortisone has been reported to delay tissue repair of experimental TM perforation in rats,^{18,19} the molecular mechanisms of delayed healing of the TM have not been described previously. In this study, induction of KGF, TGF- α , and bFGF expression was shown to be inhibited during healing of TM perforations. Moreover, the average number of BrdU-positive

cells in the TM in the glucocorticoid-treated group was significantly reduced as compared with that in the controls. Since these growth factors stimulate mitogenesis and chemotaxis for a variety of cell types, inhibition of DNA synthesis via decreased expression of growth factors as shown in this study provides a likely explanation for the delayed wound healing of the TM in glucocorticoid-treated rats.

According to studies on the process of normal TM healing in animals, there is a marked accumulation of epidermis at the edge of the perforation in the initial stage, which then proliferates to close the defect.²⁰ Epidermal migration appears to be a key factor in the healing of TM perforations.² In this study, there was no epidermal hyperplasia at the edge of the perforation in the glucocorticoid-treated rats, although there was a ridge of epidermis a small distance away from the perforation edge. This finding suggests that suppression of growth factors by glucocorticoid may contribute to the discontinuation of physiologic migration in the early stage of TM perforation healing.

Since the down-regulation of growth factors is associated with wound healing defects of the TM, the exogenous application of growth factors is expected to reverse the wound healing defects of the TM in glucocorticoid-treated rats. Therefore, topical use of these growth factors, delivered as an ear drop, might facilitate wound healing of the TM in patients receiving chronic glucocorticoid therapy. Data on KGF, TGF- α , and bFGF expression patterns in normal and impaired wound healing may provide useful information as to the most effective type of growth factor and the timing of its administration in the treatment of intractable TM wounds.

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The Congress of the ENT Section of the European Academy of Allergology and Clinical Immunology will be held in Ghent, Belgium, November 15-18, 2003, and will be accompanied on November 17-19, 2003, by the Fifth International Symposium on Experimental Rhinology and Immunology of the Nose. For further information, contact Congress Secretariat, Semico nv, Korte Meer 16, B-9000 Ghent, Belgium; telephone +32 9 233 86 60; fax +32 9 233 85 97; e-mail eaaci@semico.org or see the web site <http://www.semico.org/serin>.

MEMORIAL
DAVID G. HANSON, MD
1943-2002



On Wednesday, July 10, 2002, Dr David Hanson, 58, died of cancer at his home in La Porte, Indiana. Until his recent illnesses, Dr Hanson had been Professor and Chairman of the Department of Otolaryngology–Head and Neck Surgery at Northwestern University Medical School. He was also well known for his expertise in laryngeal and voice research.

David Hanson was born in Seattle, Washington, on November 16, 1943. He attended Wheaton College in Illinois and medical school at the University of Washington in Seattle, graduating in 1970. His residency was done at the University of Minnesota, where he also obtained a Master of Science degree. After completion of his residency in 1975, Dr Hanson served 3 years on active duty with the United States Public Health Service as a Staff Scientist at the National Institutes of Health, where he initiated and developed a program involving clinical otolaryngology within the Communicative Disorders Program.

In 1978, Dr Hanson joined the academic faculty of the Division of Head and Neck Surgery at the University of California at Los Angeles (UCLA). He served as Chief of the Section of Head and Neck Surgery at the Veterans Administration Medical Center in West Los Angeles and as Vice Chief of the UCLA Division of Head and Neck Surgery. It was

during his tenure at UCLA that Dr Hanson's interest in clinical and investigative laryngology evolved and his program of laryngeal and voice research became increasingly productive. Beginning in 1985, and continuously for 15 years until he became ill, his research was funded by the National Institutes of Health, and many contributions were made to our understanding of laryngeal physiology.

Dr Hanson came to Northwestern University as Professor and Chairman of the Department of Otolaryngology–Head and Neck Surgery in 1989 and served in that capacity until 2000. Because of illness, he stepped down from the chairmanship, but remained on the faculty until the time of his death. He was also Professor of Communication Sciences and Disorders at the Northwestern University School of Speech. He maintained an active laryngeal physiology laboratory that continued to produce numerous publications and helped train medical students, residents, and fellows. During his chairmanship, Dr Hanson revitalized the department's research laboratories. He also recruited, encouraged, and retained an excellent clinical faculty that forms the basis of today's fine department.

Dr Hanson authored 85 original research papers and 12 invited articles and book chapters. He was a member of the American College of Surgeons, the American Broncho-Esophagological Association, the American Society of Head and Neck Surgery, the American Academy of Otolaryngology–Head and Neck Surgery, the Triological Society, the American Speech-Language-Hearing Association, the American Laryngological Association, the Association for Research in Otolaryngology, and the Chicago Laryngological and Otological Society. He served on the editorial boards of 7 specialty journals. In his clinical work, he had a special interest in the care of professional voices and saw many well-known performers who came to Chicago with voice problems. A vocalist himself, Dr Hanson had a special rapport with singers who consulted him.

For a professional whose special interest was the larynx and voice, his own was soft-spoken and gentle, reflecting the kind individual who produced it. I met David Hanson the first day he came to Chicago to take over the chairmanship at Northwestern, when I was chairman at the University of Illinois. Our programs intersected at several hospitals where both of our residents served. He treated his residents kindly and was universally liked by them and his fellow

chairmen at Northwestern. When illness forced him to step down from the chairmanship in 2000, I was retiring from the University of Illinois, and I was asked to head the department at Northwestern. In the departmental office I found a quotation from Dr Hanson that reads, "I try to deal with everything I approach in life with integrity and honesty — in patient, personal and professional interactions." The person that I knew, and the department that bears his imprint,

confirm the veracity of that statement.

Dr David Hanson has left his impact on the specialty of otolaryngology—head and neck surgery, Northwestern's department, and laryngeal and voice research. He will be missed by the many friends and colleagues who have benefited from his gentle presence and by his dear wife, Terri Dangerfield Hanson.

EDWARD L. APPLEBAUM, MD

LETTER TO THE EDITOR

To the Editor:

This letter is in response to the comments made in a Letter to the Editor by Yin et al (*Ann Otol Rhinol Laryngol* 2002;111:563-5). The authors cited numerous criticisms of our work that we would like to address point by point.

As we mentioned in the article, the methods have been described in detail elsewhere in an article¹ cited by Yin et al themselves. Most of the confusion may be alleviated simply by a careful review of this paper. The endotracheal tube designed by Mermelstein et al,¹ unlike that of the tube commercially available from Xomed (Jacksonville, Florida), employs an electrode that loops away from the surface of the endotracheal tube. The wire electrode conforms to the intralaryngeal tissues, allowing flush contact with the vocal cords with slight supraglottic and subglottic extension. In our series, this tube was placed either by ourselves, or by the anesthesiologist who designed the device, under direct visualization. The special loop design of the electrode minimizes the positioning difficulties that can follow with the use of the Xomed endotracheal tube.

The electromyography (EMG) endotracheal tube that Yin et al present in their illustrations appears to represent the Xomed endotracheal tube. It requires precise placement for accurate data collection. We routinely use this tube in thyroid and parathyroid surgery for identification of the recurrent laryngeal nerve (RLN). If no EMG activity is recorded with this tube in place, the position of the tube should be checked. Most surgeons with experience with this device understand the importance of proper placement of the tube and take this step as a matter of course.

Yin et al assert that the EMG waveform produced by stimulation of the external branch of the superior laryngeal nerve (SLN) is actually produced by contraction of the thyroarytenoid muscle, as part of the efferent limb of the glottic closure reflex.² They wrongly assume that the only EMG waveform that can be recorded intralaryngeally must originate from thyroarytenoid muscle contraction and that our data can only be explained if we assume that there is a functional neurologic connection from the SLN to the thyroarytenoid muscle. Yin et al are incorrect in their assumption that there is no evidence of a connection between the SLN and the thyroarytenoid muscle. In fact, this has been demonstrated both in dogs³ and in humans.⁴

There need not be, however, a functional neurologic connection between the SLN and the thyroarytenoid muscle to produce a recordable EMG waveform. Propagation of electrical activity from the cricothyroid muscle to the electrodes of the endotracheal tube depends upon the conductive properties and the volume of the surrounding tissues, the size of the recording electrodes, and the electrodes' proximity to the cricothyroid muscle. This principle of volume conduction, with the electrical generator at a distance from the recording electrode, is the same as that used in producing an electrocardiogram. It is also the same principle that allows recording of evoked laryngeal EMG signals from an electrode placed in the esophagus.⁵⁻⁷ What Yin et al refer to as the M wave, therefore, is simply the evoked EMG waveform from the cricothyroid muscle, generated in response to electrical stimulation of the SLN.

Yin et al also criticized our failure to describe the consequences of vagal stimulation. Since the primary objective of surgery was arytenoid adduction, with reinnervation of the RLN being performed only if needed, there was no indication to perform vagal dissection in our patients. Although Yin et al raise an

interesting clinical question, it is doubtful that our hospital's office for the protection of research subjects would approve of this endeavor.

Our protocol before attempting RLN reinnervation is to wait a period of at least 9 months to allow for spontaneous recovery unless it is clear that the RLN has been sacrificed through a surgical procedure. Patient 15 in our report underwent repair of a dissecting thoracic aortic aneurysm. A phlegmon surrounding the aorta and enveloping the RLN was encountered at the time of surgery, and the RLN was knowingly sacrificed. Thus, we did not feel it necessary to wait 9 months before attempting reinnervation. We apologize for not clarifying this in the report.

The sole purpose of the article was to report our findings that in 14 of 15 patients with a vocal cord paralysis, no EMG waveform could be elicited with stimulation of the ipsilateral RLN of the paralyzed side. This indicated to us that no meaningful electrical activity was propagating along the RLN. Only 1 patient with a vocal cord paralysis demonstrated EMG activity, which indicated the possibility of synkinesis. In this patient, the RLN was not reinnervated.

The concept that synkinesis occurs commonly in patients with vocal cord paralysis^{8,9} has been heretofore embraced strongly by the otolaryngology community with a relative paucity of supporting data. Our paper challenges the concept of synkinesis in the RLN as a common finding in patients with vocal cord paralysis. It is relatively easy to confirm or to refute our conclusions by simple repetition of our procedure, and we invite our colleagues to report their findings in this regard.

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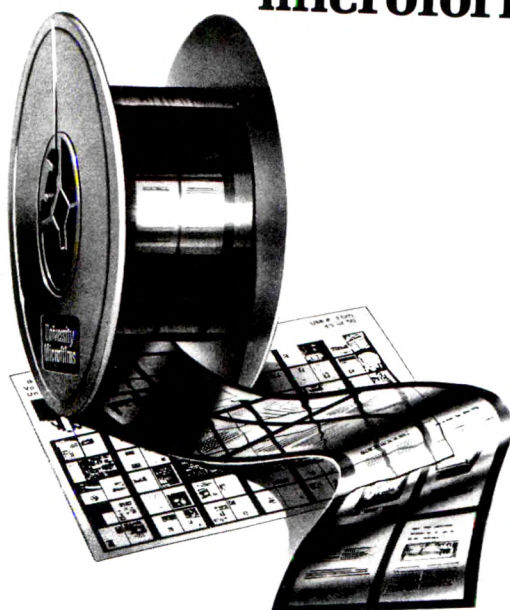
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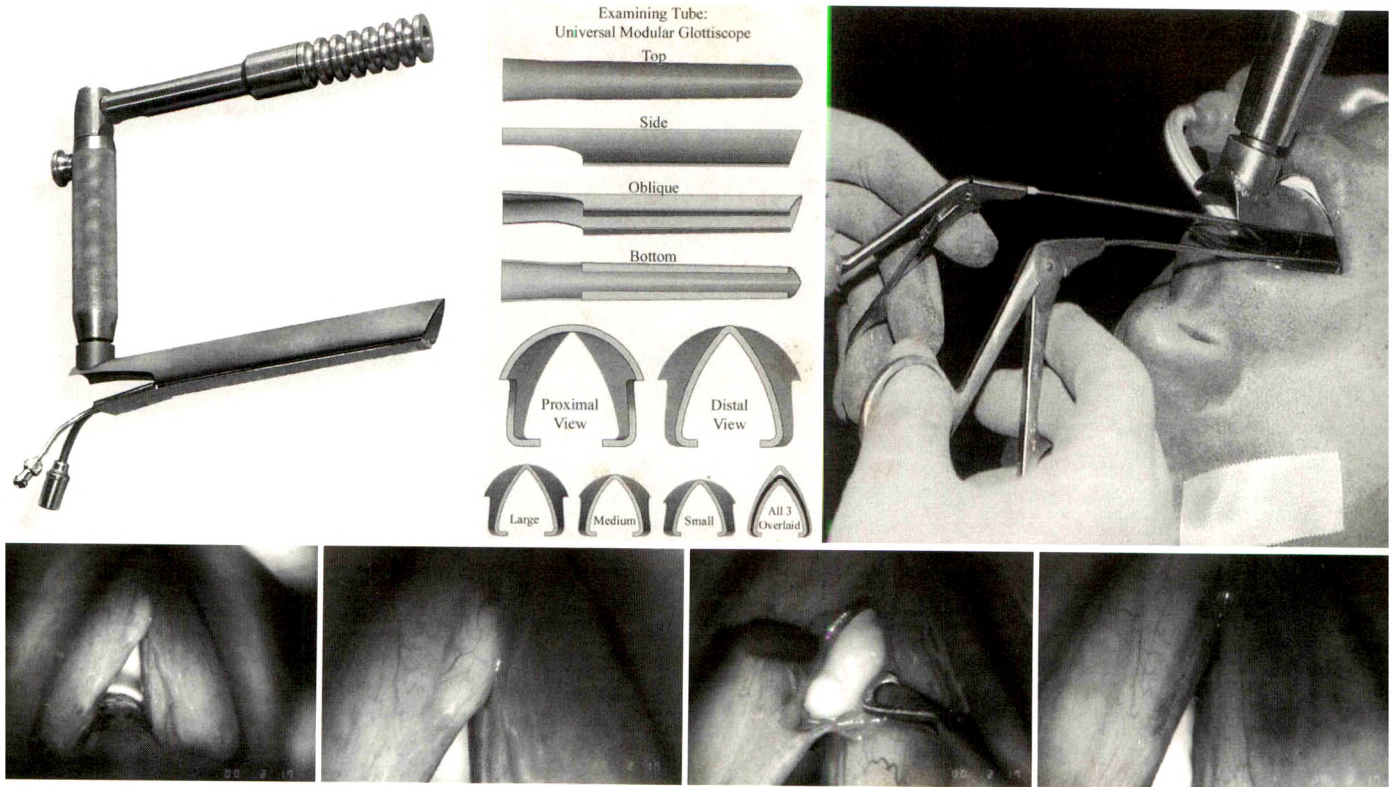


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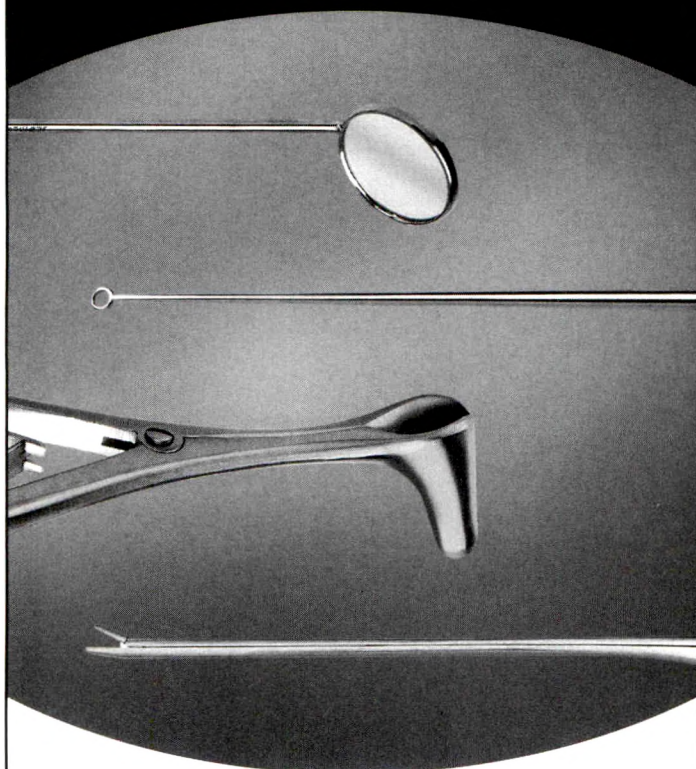


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36th Annual IOWA Head and Neck Cancer & Reconstructive Surgery Course

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For further information regarding these courses, contact Henry T. Hoffman, MD, Dept of Otolaryngology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242 • 319-356-2166 • Fax 319-356-4547

SURVIVIN EXPRESSION IN JUVENILE-ONSET RECURRENT RESPIRATORY PAPILLOMATOSIS

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IOWA CITY, IOWA

RICHARD J. H. SMITH, MD

IOWA CITY, IOWA

DAVID L. SCOTT, MD

IOWA CITY, IOWA

NANCY M. BAUMAN, MD

IOWA CITY, IOWA

Recurrent respiratory papillomatosis (RRP), caused by the human papillomavirus, is characterized by unregulated growth of wartlike neoplasms on laryngeal mucosa. Apoptosis is important in normal cellular homeostasis, and dysregulation of this process is thought to govern the behavior of certain neoplasms. This study evaluates the expression of several pro-apoptotic and anti-apoptotic factors in papillomas of patients with RRP, with a specific interest in survivin, a cell cycle-regulated anti-apoptotic factor. Three anti-apoptotic and 6 pro-apoptotic messenger RNA (mRNA) species were quantified by ribonuclease protection assay in 11 RRP papilloma specimens and 5 normal laryngeal specimens. Anti-apoptotic and pro-apoptotic mRNA ratios were quantified by normalizing to the ribosomal protein L32 and compared between specimens. Protein expression of survivin in tissue samples was also evaluated. The mean ($\pm$ SD) expression of survivin was almost fivefold greater in the RRP papillomas than in normal tissue ($14.2\% \pm 2.5\%$ versus $3.0\% \pm 0.8\%$ of L32, $p = .003$). The RRP specimens also had greater expression of XIAP, Fas, and p53 than did the normal tissue. Survivin protein was differentially expressed in the papilloma specimens, and was greatest in a papilloma that underwent malignant transformation. Survivin was absent in all normal laryngeal tissue tested. Apoptotic factors in general appear to be up-regulated in papillomatous tissue as compared to normal laryngeal tissue and may suggest a higher proliferation rate and cell turnover. Survivin is abundant in papillomas and absent in normal laryngeal tissue. Dysregulation of apoptosis as determined by abnormal expression of anti-apoptotic factors like survivin and XIAP probably favors papilloma growth and survival. Such factors may represent potential targets in the treatment of this disease.

KEY WORDS — apoptosis, human papillomavirus, recurrent respiratory papillomatosis, survivin, XIAP.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is a potentially devastating disease that afflicts 4.3 in 100,000 infants and children.¹ The disease is caused by the human papillomavirus (HPV) and is characterized by the appearance of wartlike excrescences in the larynx that cause hoarseness and/or airway obstruction. The growth of these lesions may be relentless, and to date, there is no cure for RRP.

Although vertical transmission may occur in utero by hematogenous spread or by direct extension, the HPV is more likely transmitted to infants during delivery through a vaginal canal harboring an active or latent infection. A common viral infection of the cervix, HPV is present in an active form or a latent, asymptomatic form in 10% to 25% of women of childbearing age.² Approximately 6,000 children are currently afflicted with active RRP.¹ This relatively low number of patients with active disease suggests a low rate of vertical transmission. Of the known 90

different types of HPV, only HPV type 6, type 11, and occasionally type 16 appear to cause RRP.³

Papillomas in patients with RRP tend to develop at the junction of squamous and ciliated respiratory epithelia and are most commonly found along the true and false vocal cords. Although papillomas may not appear in the remaining respiratory mucosa lining the aerodigestive tract, the HPV genome is often harbored in normal-appearing cells in patients with RRP.

Surgical extirpation of the laryngeal papillomas is a temporizing measure to provide children with a serviceable voice and to prevent asphyxiation. Despite surgical removal, the papillomatous growths typically recur within weeks or months. Spontaneous remission occurs in most children, but the age of remission is highly variable and unpredictable. Some children never enter remission and carry the disease into adulthood. These children are at greater risk of developing pulmonary lesions with the potential to

From the Departments of Otolaryngology-Head and Neck Surgery (Poetker, Smith, Bauman) and Surgery (Sandler, Scott), The University of Iowa Hospitals and Clinics, Iowa City, Iowa. Dr Poetker is currently in the Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, Milwaukee, Wisconsin.

Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 14-15, 2001.

CORRESPONDENCE — Nancy M. Bauman, MD, Dept of Otolaryngology, The University of Iowa Hospitals and Clinics, 200 Hawkins Dr, Iowa City, IA 52242.

undergo malignant degeneration.³

For unknown reasons, the growth rate of papillomas is extremely variable; some patients require monthly removal of the lesions, whereas other patients with active disease require only 1 or 2 annual procedures. It is possible that alterations in the process of apoptosis, or programmed cell death, may account for the variable growth patterns seen in such patients. Disruption of the balance between pro-apoptotic and anti-apoptotic factors may affect differential cell growth and the development of neoplasms.⁴

Survivin is an anti-apoptotic factor that is expressed in normally developing fetal tissues and also in most tumors, but is rarely expressed in differentiated, senescent, normal tissues.⁵⁻¹² Survivin is also present in premalignant lesions such as Bowen's disease and hypertrophic actinic keratosis.¹³ Because of survivin's differential expression in highly proliferative cells and its unique association with the G2/M (proliferative) phase of the cell cycle, this apoptosis inhibitor may represent an early step toward tumor progression.

The effect of HPV infection on cellular apoptosis and on expression of apoptotic factors is not clearly defined. Dysregulation of apoptosis may play a role in the pathogenesis of RRP whereby exuberant epithelial proliferation induces the development of papillomas. The purpose of this study was to evaluate expression of apoptotic factors, particularly survivin, in papilloma specimens and nondiseased laryngeal mucosa or epithelium.

MATERIALS AND METHODS

Tissue Specimens. Seventeen frozen tissue specimens were obtained from patients at The University of Iowa Hospitals and Clinics. Expression of multiple messenger RNA (mRNA) species was compared between laryngeal papilloma specimens (n = 11) and normal-appearing laryngeal mucosa (controls; n = 5). Control specimens were obtained from patients without RRP who were undergoing other procedures (supraglottoplasty, n = 2; laryngectomy, n = 1) and from cadaver specimens (n = 2). A single malignant specimen was obtained after degeneration of a papilloma. Tissue harvested for analysis was approved by the Institutional Review Board.

Ribonuclease Protection Assay. The RiboQuant multi-probe protection assay system (PharMingen, San Diego, California) is a highly sensitive and specific method for simultaneous detection and quantification of multiple mRNA species. A customized template of human apoptosis probe sets (custom human template set; PharMingen) was used to detect expression of XIAP, survivin, Fas, p53, BID, DR5

(TRAIL-R2), DR4 (TRAIL-R1), Bax, Bcl2, L32, and GAPDH.

Total RNA was isolated from the tissue specimens with Trizol (Gibco Life Technologies, Rockville, Maryland). The ³²P-labeled antisense RNA probe was added, in excess, to 14 to 16 µg of target RNA. ³²P-labeled antisense RNA was transcribed by means of T7 RNA polymerase (PharMingen) and [α-³²P]UTP (Amersham Life Science, London, England). A 1:417 dilution of ribonucleases (RNases; RNase A:T1 mixture; PharMingen) was added to the solution for 45 minutes at 30°C to digest the unhybridized probe and target RNA. The RNases were inactivated, and the protected, double-stranded RNA pellets were dried, resuspended in 5 µL of 1× loading buffer (PharMingen), and electrophoresed on a 5% polyacrylamide–8 mol/L urea gel. The polyacrylamide-urea gel was dried on blotting paper for 1 hour at 80°C. The labeled probes were then quantified by autoradiography by exposing the blotting paper to Bio Max Film (Kodak, Rochester, New York) with an intensifying screen (Kodak) for 20 hours at –80°C.

Radio-Analytic Imaging. For comparative analysis, we quantified mRNA species expression as a percentage of the ribosomal protein L32 mRNA expression. The radioactivity of each band in the sample was quantified, standardized, and compared to the L32 expression of the sample. The radioactivity of each template was quantified directly from the gel for 1,000 minutes by a radio-analytic imaging system (Ambis 4000, Ambis Inc, San Diego) equipped with Ambis QuantProbe software version 3.0. Net counts were obtained from each template (band) including L32, and the radioactivity was expressed as a percentage of the L32 activity.

Western Blot for Protein Expression. For confirmation of protein expression, the standard technique of Western blot was performed. Briefly, tissue lysate proteins were isolated by macerating tissue biopsy specimens and solubilizing cells with RIPA buffer and protease inhibitors. The protein content was quantified by the Lowry protein assay.¹⁴ Isolated proteins (120 µg) were diluted in Laemmli buffer and separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under nonreducing conditions. Subsequently, proteins were electrophoretically transferred to a nitrocellulose membrane at 210 mA for 3 hours. Membranes were then blocked in TBS plus 5% skim milk for 1 hour at room temperature or overnight at 4°C. After washes in TBS with 0.3% Tween-20, the membranes were then incubated for 1 hour at room temperature with rabbit polyclonal antibody to survivin (20 µg/mL; supplied by Dario Altieri, Yale College of Medicine, New Haven, Con-

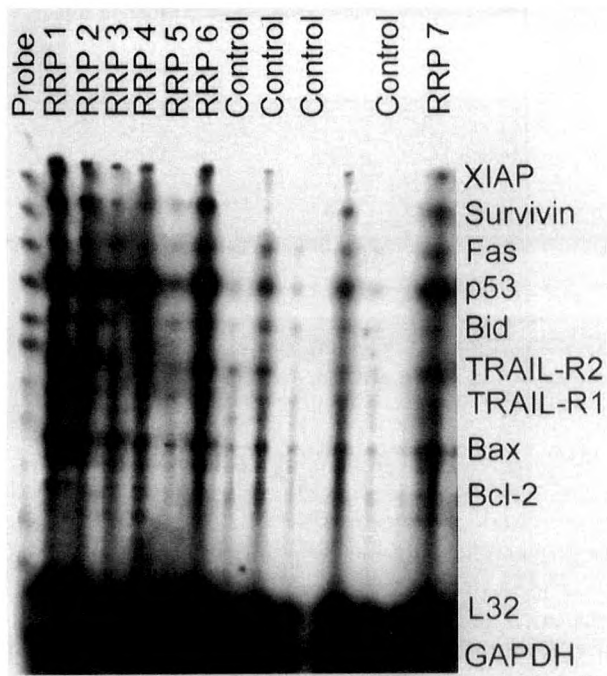


Fig 1. Example of ribonuclease protection assay (RPA) autoradiographic plate showing bands marking various messenger RNA species detected in several specimens of recurrent respiratory papillomatosis (RRP) and normal tissues.

necticut). Again after washing, the membranes were incubated with a 1:2,500 dilution of horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody at room temperature for 1 hour. Bands were viewed by a chemiluminescence reaction with the ECL chemiluminescence system (Amersham, Arlington Heights, Illinois).

Data Analysis. Apoptotic mRNA expression was determined and compared among papillomas and normal tissue specimens by Student's *t*-test.

RESULTS

Ribonuclease Protection Assay. Apoptotic mRNA from tissue specimens was quantified as a ratio of their own ribosomal protein L32, and expression in RRP samples ($n = 11$) was compared to that in normal laryngeal tissue samples ($n = 5$; Fig 1). The mean ($\pm$ SD) survivin expression in the RRP samples was $14.2\% \pm 2.8\%$ of L32 expression, whereas the mean survivin expression in normal laryngeal tissue was only $3.0\% \pm 0.8\%$ of L32 ($p = .003$). The differential mRNA survivin expression in papillomas ranged from 5% to 35% of L32 expression, whereas the range in normal tissue was 1% to 5%. Similarly, XIAP, another inhibitor of apoptosis within the same family as survivin, was expressed at $14.2\% \pm 4.3\%$ of L32 in RRP specimens, but only at $0.4\% \pm 0.2\%$ of L32 in normal controls ($p = .009$; Fig 2). Expression of Bcl-2, another inhibitor of apoptosis unre-

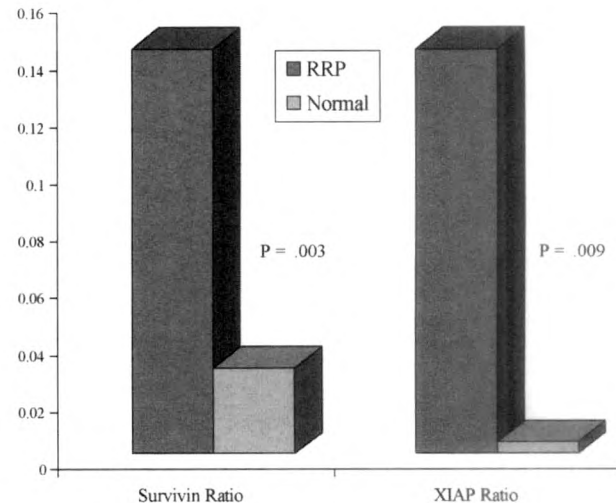


Fig 2. RPA results of survivin and XIAP expression in normal and RRP specimens. Messenger RNA expression is quantified as percentage of L32 expression in each sample. *p* Values shown as calculated by Student's *t*-test.

lated to the survivin family, did not differ between papillomas and controls ($p = .6$).

The mRNA expression of several pro-apoptotic factors was also up-regulated in papillomas, perhaps reflecting a higher rate of turnover and an increased rate of proliferation of cells. Fas expression in the RRP samples was $12.0\% \pm 2.3\%$, as compared with $4.8\% \pm 0.9\%$ in the normal tissues ($p = .01$). The RRP tissues had a mean p53 expression of $40.2\% \pm 9.4\%$, whereas normal tissues had a mean p53 expression of $9.8\% \pm 2.5\%$ ($p = .02$). The ratio of survivin expression to Fas expression in the RRP samples was 0.87, and this ratio in the control specimens was 0.66 ($p = .23$).

Survivin expression in the pulmonary tumor that had undergone malignant transformation from a papilloma was 42.8% of L32 expression, but was only 7.6% in the nondegenerated laryngeal papilloma obtained from the same patient several years earlier.

Western Blot Analysis. Western blot analysis confirmed the expression of survivin protein in the RRP specimens, whereas no survivin protein was detected in any of the non-RRP samples tested. Differential expression of survivin between samples was again noted (Fig 3).

DISCUSSION

Apoptosis is an essential process critical for normal development and cellular homeostasis, which depends on a balance of pro-apoptotic and anti-apoptotic factors. There are many different triggers and stimuli that lead to apoptosis; however, they all converge to a central apoptotic pathway in which cysteine proteases, called caspases, are activated.¹⁵ The apoptotic mediators stimulate the activity of initia-

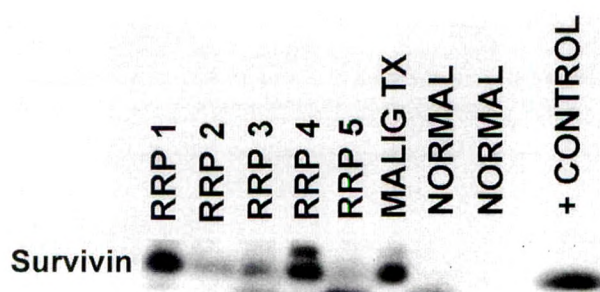


Fig 3. Western blot analysis of survivin expression in several RRP tissue samples (RRP 1 through RRP 5), normal tissue samples (NORMAL), single specimen following malignant transformation (MALIG TX), and positive control (+ CONTROL). Note differential expression of survivin in RRP specimens and absence of survivin in normal laryngeal tissue tested. Equal amounts of protein were loaded in each lane, and numbers assigned to each specimen do not correlate with numbers in Fig 1.

tor caspases that then go on to activate effector caspases.¹⁵ The effector caspases induce the cellular changes that lead to cell death.¹⁵

All cells have regulatory proteins that protect the cell from the premature activation of the apoptotic pathway in order for the cell to survive and to perform its specific function. Multiple families of proteins, including the inhibitor of apoptosis protein (IAP) family and the Bcl-2 family, are inhibitors of apoptosis that play a role in regulating normal cellular homeostasis. Overexpression of a regulatory protein, or perhaps expression at a critical point of the cell cycle, may lead to cellular malfunction and disease development.

Survivin is a member of the IAP family, and has been described as having a topographic structure similar to that of microtubule-associated proteins.¹⁶ Survivin is thought to be a transcription-regulated protein, expressed in the G2/M phase, and its function is believed to be associated with mitotic spindle stabilization during cell division.¹⁶ Survivin is expressed in fetal tissue and is a common feature of malignant cells, but is notably absent in most mature, differentiated tissues. Survivin expression is associated with tumor aggressiveness and is thought to permit aberrant proliferation through the cell cycle by protecting the cell from apoptosis. Survivin is also shown to inhibit specific caspases of the apoptotic pathway.^{16,17}

Human papillomavirus infections have long been

associated with carcinoma. The role of the HPV infection is not entirely known; however, it is widely accepted that HPV genes E6 and E7 are responsible for disruption of cell cycle control. The gene product of E6 has been shown to bind to p53 and cause its destruction through ubiquitin-mediated proteolysis, thus inhibiting the regulatory effects of this protein. The E7 gene product has been shown to alter the phosphorylation state of the Rb gene product, inactivating the protein.^{18,19} The pathogenicity of the HPV strain may be related to the affinity of the individual strain's E6 and E7 proteins for their respective targets.¹⁹

By enhancing an anti-apoptotic factor, such as survivin, viral infection of a cell may enhance survival of both the virus and its host cell. This study demonstrates a significant increase in the expression of the anti-apoptotic factor survivin and IAP in papilloma specimens sampled from patients with RRP. We have also demonstrated a tenfold increase in survivin expression in a single sample of malignant papilloma tumor over the mean survivin expression of control laryngeal tissue, with a sixfold increase in expression over its premalignant state. Although abundant anti-apoptotic Bcl-2 gene expression has been reported by other investigators using reverse transcription-polymerase chain reaction,²⁰ we did not find Bcl-2 mRNA expression to be increased in papilloma specimens as compared to normal control mucosa.

The extent and growth rate of papillomas vary markedly among patients with RRP. This variation may be dependent on both the proliferation rate of the papilloma and the ability of the immune system to suppress further growth. Survivin expression in infected cells may not only enhance aberrant proliferation of the host cell, but it may also protect the cell from immune-mediated destruction. Differential expression of survivin was noted in the papillomas sampled, and although speculative, it is possible that survivin expression may correlate with disease activity. Papillomas form from normal tissues after infection with a viral catalyst. These benign neoplasms may continue to grow at a stable rate, may degenerate to a malignancy, or may spontaneously regress. Dysregulation of cellular apoptosis by HPV infection could play a critical role in defining the ultimate outcome of this disease. Specific apoptotic mediators that are aberrantly expressed in papillomas may act as novel therapeutic targets.

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AGE-RELATED ALTERATIONS IN MYOSIN HEAVY CHAIN ISOFORMS IN RAT INTRINSIC LARYNGEAL MUSCLES

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Deficits in voice and swallowing are found in the elderly, but the underlying neuromuscular mechanisms are unclear. A potential mechanism may be denervation-induced muscle fiber transformation to a slower-contracting type of muscle fiber. This study examined young, old, and denervated rat laryngeal muscles (lateral thyroarytenoid, lateral cricoarytenoid, and posterior cricoarytenoid) to examine differences in myosin heavy chain (MHC) composition. Results of sodium dodecyl sulfate–polyacrylamide gel electrophoresis analyses indicated that all muscles were composed predominately of type IIB MHC. With aging and denervation, type IIB was reduced and type IIX, a slower-contracting isoform, was increased in the lateral thyroarytenoid and lateral cricoarytenoid muscles. In the posterior cricoarytenoid muscle, the MHC composition was relatively unchanged. These findings suggest that aging may affect laryngeal adductory function by altering muscle fiber type composition to a slower-contracting isoform, in a manner similar to that observed with denervation.

KEY WORDS — aging, lateral cricoarytenoid muscle, myosin heavy chain, posterior cricoarytenoid muscle, thyroarytenoid muscle.

INTRODUCTION

Deficits in voice and swallowing are frequently found in the elderly.¹⁻⁵ However, the underlying neuromuscular mechanisms of age-related dysphonia and dysphagia are unclear, because few investigations have directly addressed the upper aerodigestive tract. Most work on aging has focused on limb musculature. Direct study of cranial muscles is technically difficult, particularly within the larynx, because of the small size and limited access. It is necessary, however, to study laryngeal muscles directly, because there are substantial differences between limb and laryngeal muscles in structure and innervation ratios.⁶⁻⁹

Information available from limb muscle studies is indicative of marked changes in the contractile properties of senescent muscle, including reductions in peak twitch tension and peak tetanic tension, and increased time to peak and relaxation time values.¹⁰ Aging does not affect all muscles or muscle fiber types equally,¹¹ in that greater age-related atrophy has been found in fast-twitch (type II) fibers relative

to slow-twitch (type I) fibers.^{12,13} Because of the rapidly contracting nature of laryngeal muscles, age-related changes in the intrinsic muscles of the larynx are likely. In fact, in aged thyroarytenoid muscle, there have been reports of slower contractions with reduced amplitude and slower recovery from fatigue.^{14,15} These changes in laryngeal muscle contractile properties correspond to structural observations of decreased type II and increased type I muscle fiber composition with increasing age,¹⁶ which suggest muscle fiber transformation to a slow-contracting fiber type.

Myosin heavy chain (MHC) is the primary component of the thick filament, is the predominant protein in skeletal muscle, and is expressed in a tissue-specific and developmentally regulated manner.¹⁷ The maximum contraction speed of a given muscle fiber is determined by particular MHC isoforms, which can be identified via a variety of enzyme histochemical, immunohistochemical, and gel electrophoresis techniques. Five MHC isoforms have been identified in intrinsic laryngeal muscles in human

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and animal models, including types I (slow), IIA (fast), IIB (fast), IIX or IID (fast), and IIL (super-fast).^{18,19}

In laryngeal muscle, alterations in MHC isoforms have been observed after muscle injury,²⁰ nerve section,²¹ chemodenervation,²² and thyroid hormone imbalance.²³ With aging, alterations in MHC composition have also been reported in jaw and limb muscles,²⁴ such that type IIB composition tends to decrease while type IIX increases.²⁵ Interestingly, it has been suggested that MHC composition in denervated laryngeal muscle is characterized by a transformation to a more slowly contracting isoform.²² Because of the slower contraction and recovery times found in senescent muscle, it is reasonable to expect that aged muscle might also manifest denervation-like changes to a slower MHC isoform. Accordingly, it is our hypothesis that alterations observed in the morphology or physiology of aged laryngeal muscle reflect a natural denervation process. The goal of this study was to examine aged laryngeal muscle in a rat model to determine whether aged muscles manifested alterations in MHC composition suggestive of denervation.

MATERIALS AND METHODS

Experimental Animals and Surgical Procedures. Six young (9-month-old) and 5 old (24-month-old) Fischer 344/Brown Norway hybrid (F1 hybrid) rats were obtained from a National Institute on Aging animal colony. Their body weights averaged 398 g (SD, 17 g) for the young rats and 591 g (SD, 62 g) for the old rats. The old animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (9 mg/kg) and euthanized with an intracardiac injection of 7.2% chloral hydrate. Whole larynges were immediately harvested and frozen in liquid nitrogen. In the young rats only, the left recurrent laryngeal nerve was sectioned to create a unilateral left-sided denervation of the laryngeal muscles. In prior work, we determined that reinnervation effects in rat laryngeal muscle could be observed 2 to 4 weeks after denervation. Because of our interest in studying denervation-reinnervation processes in this investigation, we waited 4 weeks after the denervation procedure to euthanize the animals and harvest the larynges. As a control, left-sided laryngeal paralysis was confirmed by means of a rat microlaryngoscopic technique before the animals were euthanized.²⁶ Electromyographic data on the paralyzed side were not collected because of our assumption, based on observations of neuromuscular junction changes in another study, that reinnervation processes were under way at this time point.²⁷ To view the vocal folds, we placed the anesthetized

animal into a malleable lead operating platform in a near-vertical position with the mouth held open. A custom-made wire laryngoscope was then inserted into the mouth, and the animal's position was adjusted to maximize the view of the vocal folds under a Zeiss surgical microscope. After unilateral vocal fold paralysis was visually confirmed, the animals were euthanized and their larynges were removed and frozen.

With a cryostat, 20- and 60- μ m serial coronal sections were made of laryngeal blocks for study of the lateral thyroarytenoid (LTA) muscle and lateral cricoarytenoid (LCA) muscle, and serial axial sections were made for the posterior cricoarytenoid (PCA) muscle. All muscle sections were mounted on microscope slides and stored in a -70°C freezer. In the old rats, analyses encompassed laryngeal muscles on 1 side of the larynx, and in the young animals, both the normal and denervated sides were studied. In this manner, MHC composition was examined in 3 distinct groups of muscle samples: young, old, and denervated.

Muscle Section Staining. The 20- μ m-thick laryngeal sections were stained by a standard hematoxylin and eosin protocol for identifying the LTA, LCA, and PCA muscles. This procedure was performed as a control to ensure that muscle samples identified in the thicker sections (60 μ m) were in fact from the 3 target muscles.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Specimens from individual muscles for each animal were processed separately. The 60- μ m sections were soaked for 15 minutes in an ice-cold relaxing solution (mmol/L: KCl, 100; imidazole, 20; MgATP, 4; EGTA, 2; free Mg^{2+} , 1; pH 7.0 at 22°C).²⁸ Small bundles of muscle fibers were dissected from each muscle section with a stainless steel needle under a stereomicroscope. Each specimen was placed in 20 μ L of sample buffer (15% glycerol, 1% sodium dodecyl sulfate, 14 mmol/L Tris, 3.4 mmol/L dithiothreitol, 0.5% leupeptin, 0.05% bromophenol blue). The muscles were centrifuged at 14,000g for 1 minute and homogenized in an ultrasonic bath. The sample buffer was diluted up to 10 times to adjust the protein amount. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed with a 0.75-mm-thick 6% acrylamide/30% glycerol separating gel (18 $\times$ 16 cm) and a 4% acrylamide/30% glycerol stacking gel. The running conditions were set at 275 V and 20 mA for 24 hours at 10°C . The gel was stained with 1 mmol/L silver nitrate and developed to visualize protein bands.

Data Analysis. Individual silver-stained gels were digitally imaged on a flatbed scanner with a transpar-

PERCENTAGE (MEAN $\pm$ SD) AND P VALUE ON POST HOC TESTS OF EACH MYOSIN HEAVY CHAIN

	Type IIA	Type IIX	Type IIB	Type IIL	Type I
Lateral thyroarytenoid muscle					
Young	0	0	99.7 $\pm$ 0.5	0.3 $\pm$ 0.5	0
Old	0	11.0 $\pm$.8	88.0 $\pm$ 8.0	1.0 $\pm$ 1.2	0
Denervated	0	22.3 $\pm$ 4.8	76.6 $\pm$ 6.1	1.1 $\pm$ 2.3	0
p Value on post hoc tests (Scheffe)					
Young versus old	N/A	.01	.01	NS	N/A
Young versus denervated	N/A	<.0001	<.0001	NS	N/A
Old versus denervated	N/A	.008	.02	NS	N/A
Lateral cricoarytenoid muscle					
Young	6.4 $\pm$ 7.7	45.0 $\pm$ 5.2	46.0 $\pm$ 11.1	2.5 $\pm$ 4.2	0.1 $\pm$ 0.2
Old	18.5 $\pm$ 8.6	65.7 $\pm$ 6.7	10.8 $\pm$ 9.0	5.0 $\pm$ 3.6	0
Denervated	3.6 $\pm$ 3.8	71.6 $\pm$ 12.3	24.0 $\pm$ 11.7	0.8 $\pm$ 0.8	0
p Value on post hoc tests (Scheffe)					
Young versus old	.04	.006	.0004	NS	.006
Young versus denervated	NS	.0005	.01	NS	NS
Old versus denervated	.01	NS	NS	NS	N/A
Posterior cricoarytenoid muscle					
Young	7.1 $\pm$ 1.8	11.2 $\pm$ 2.0	78.8 $\pm$ 3.8	2.9 $\pm$ 0.7	0
Old	11.3 $\pm$ 4.3	12.1 $\pm$ 0.8	73.5 $\pm$ 5.4	3.0 $\pm$ 1.0	0
Denervated	6.5 $\pm$ 3.2	61.6 $\pm$ 5.6	27.6 $\pm$ 3.9	4.3 $\pm$ 2.4	0
p Value on post hoc tests (Scheffe)					
Young versus old	NS	NS	NS	NS	N/A
Young versus denervated	NS	.0001	.0001	NS	N/A
Old versus denervated	NS	.0001	.0001	NS	N/A

N/A — not applicable; NS — not significant.

ency system and analyzed densitometrically by computer-assisted image analysis (Scion Image 1.62). The percentage of each MHC isoform in each column of the gel was calculated by using the density waveform as the area under the curve for a particular MHC band over the total area for all MHC bands in that column. In this manner, the relative MHC composition for each muscle was calculated. One-way analysis of variance followed by Scheffe's post hoc test was performed to compare the MHC composition expressed for each muscle as a function of age or denervation group.

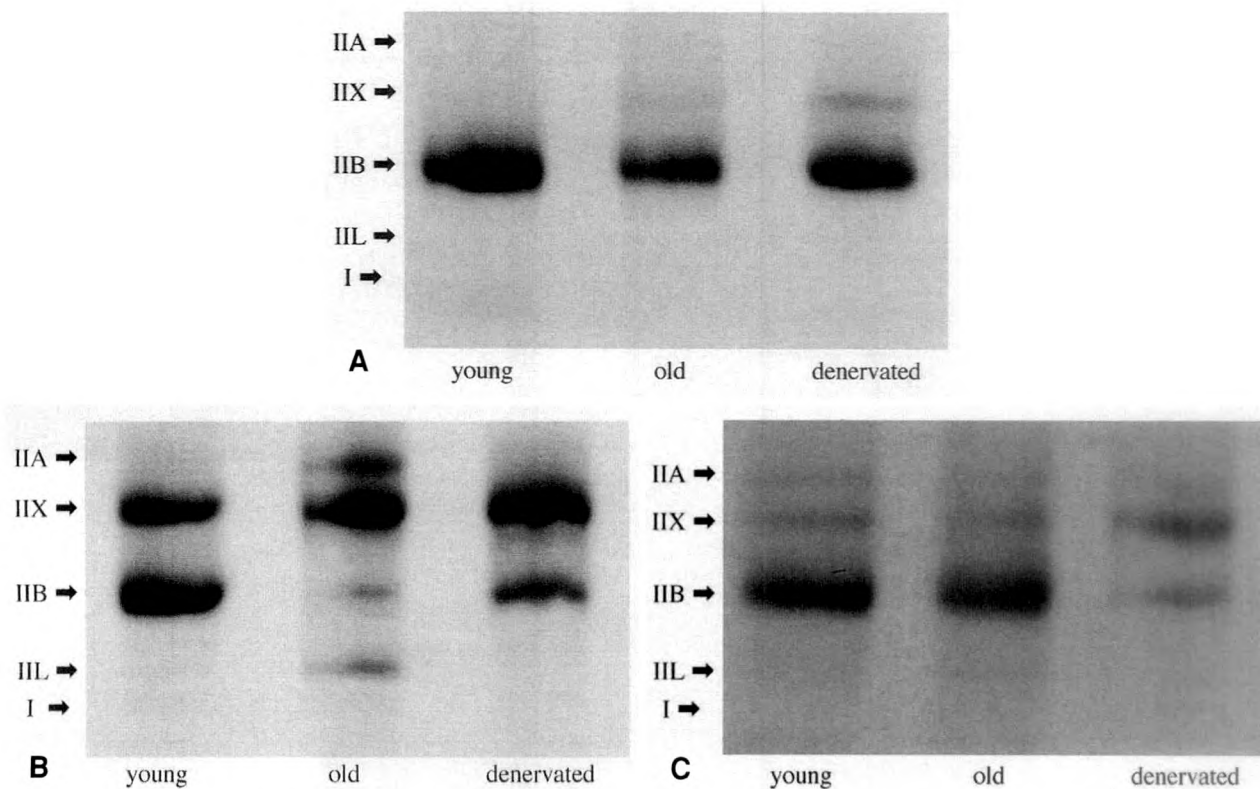
RESULTS

Lateral Thyroarytenoid Muscle. As shown in the Table and the Figure, A, significant differences in MHC composition were found among the young, old, and denervated muscles. In this adductory muscle, the proportion of type IIB MHC was significantly and progressively reduced in the old and denervated muscles relative to the young muscles. In contrast, the proportion of type IIX MHC was observed to increase significantly in the denervated and old muscles relative to the young. Significant differences were found among all 3 muscle groups on post hoc tests, such that the old muscles evidenced a significantly larger proportion of type IIX MHC than the

young, and the denervated muscles manifested a significantly larger proportion than the old. This progressive increase of type IIX MHC was the opposite of the progressive decrease noted for the type IIB MHC with aging and denervation. No type IIA or type I MHC was detected in any of the LTA muscles for any group. No significant differences in the presence of the superfast isoform, type IIL, were found across age or denervation groups.

Lateral Cricoarytenoid Muscle. The proportion of type IIB MHC was significantly reduced in the old muscles relative to the young muscles, whereas type IIX MHC proportions were significantly increased (see Table and Figure, B). Type IIA MHC proportions in the old muscles were significantly larger than those in the young and denervated muscles. No significant differences in type IIL were found across age or denervation groups. A small amount of type I MHC was found in the LCA muscle.

Posterior Cricoarytenoid Muscle. Data for this abductory muscle are found in the Table and the Figure, C. Although there were no differences in type IIB and type IIX MHC composition in the young group versus the old group, the proportions of these isoforms were significantly altered in the denervated muscles. Specifically, the type IIB proportion was



Changes in myosin heavy chain isoforms in muscles on representative gels from sodium dodecyl sulfate–polyacrylamide gel electrophoresis. **A)** Lateral thyroarytenoid muscles. Myosin heavy chain types IIX, IIB, and IIL were detected. No type IIA or type I was detected. Proportion of type IIB was significantly reduced in old and denervated muscles relative to young muscles, while proportion of type IIX was increased significantly. **B)** Lateral cricoarytenoid (LCA) muscles. Types IIA, IIX, IIB, and IIL and small amount of type I were detected. Proportion of type IIB was significantly reduced in old muscles relative to young muscles, while type IIX proportions were increased. **C)** Posterior cricoarytenoid muscles. Types IIA, IIX, IIB, and IIL and small amount of type I were detected. No significant differences were noted between young and old muscles in isoform proportion. However, in denervated muscles, significant increase in type IIX and reduction in type IIB were found in comparison with young muscle. (At IIB, mark above myosin heavy chain band for young animals is artifactual.)

seen to be significantly reduced, while type IIX MHC had increased. This is the same pattern of MHC transformation noted for denervated LTA and LCA muscles. No change was detected in the proportion of type IIA or type I MHC isoforms across age or denervation groups. Very little type IIL MHC was detected in any of the PCA muscle samples. However, an increase in the proportion of the superfast type IIL isoform was detected in the old muscles relative to the young and denervated samples.

DISCUSSION

The larynx facilitates the important biological functions of airway protection and voice production, and appears to undergo significant functional changes throughout the life span. With regard to airway protection, weakness and atrophy of the laryngeal muscles may contribute to the large numbers of elderly persons with dysphagia and associated deficits in general health, nutrition, and quality of life. Despite this significant clinical problem, however, there has been limited research into age-related muscular

changes within the larynx. The direct study of laryngeal muscles is important toward the goal of developing effective interventions for elderly persons, or effective preventive measures.

The goal of this study was to evaluate putative changes in laryngeal muscle fiber type, with the hypothesis that the changes observed in the aging larynx might reflect a level of neuromuscular disorganization consistent with a denervation-reinnervation state. In general, the pattern of age-related alteration in MHC composition found in rat laryngeal muscles supported this hypothesis. Specifically, in the LTA muscle, a progression of alteration in type IIB and type IIX MHC composition from young to old to denervated muscle was found, such that type IIB fiber composition decreased and type IIX increased with aging and denervation. This pattern of MHC transformation was also found in the other adductory muscle, the LCA, and has been noted by others in aged limb muscles.²⁵ No change in type I MHC composition was found, but type IA MHC increased in old and denervated LCA muscles, relative to young.

The superfast isoform, type IIL, which has been identified in laryngeal muscles innervated by the recurrent laryngeal nerve and in extraocular muscles,^{18,29} is presumed to contribute to the very rapid contraction times noted for these muscles. In our study, we did not find a consistent pattern of alteration in the type IIL MHC proportion across muscles, although an increase in the old and denervated PCA muscle groups was noted.

In general, the speed of muscle fiber contraction can be characterized in terms of maximum shortening velocity ($V_{\max}$), which is closely related to MHC composition within a muscle.³⁰ Specifically, the $V_{\max}$ in type I muscle fibers is lower than that in type II muscle fibers. Within the type II category, type IIB muscle fibers have the highest $V_{\max}$, followed by type IIX and type IIA, which appear to have similar shortening velocities.^{31,32} Accordingly, a reduced proportion of type IIB MHC and an increased proportion of type IIX MHC in aged LTA and LCA muscles suggests a transformation to a slower isoform, as reported previously for denervated muscle.²⁵ These findings may explain the reductions in muscle contraction times reported in aged laryngeal muscles.^{14,15}

The regulation of MHC isoform expression is not clearly understood, but involves complex interactions among multiple intracellular signaling pathways.³³ Whereas electrical activation can transform MHC isoforms from slow to fast, reduced neuromuscular activity is associated with a reverse transformation process, from fast to slow MHC.³³ However, the rate and extent of transformation are highly dependent upon the animal species studied and the type of manipulation performed. In the rat larynx, denervation via mechanical or chemical means has typically resulted in an increase in type IIX MHC.^{21,22} Our results are consistent with these findings, in that increased type IIX MHC proportions were found in all 3 laryngeal muscles with denervation, and in the aged LTA and LCA muscles. These results reinforce our hypothesis that aging may represent a natural denervation process in the intrinsic laryngeal muscles, which may lead to a transformation to more slowly contracting muscle fiber types. It has been suggested by work in rat medial gastrocnemius that the mechanism for this change may be denervation of fast-contracting muscle fibers, with reinnervation via axonal sprouting from more

slowly contracting fibers.³⁴

Muscle disuse, which may be better termed as “reduction” or “alteration” in use,³⁵ has been proposed as a cause of muscular atrophy observed in the aged. This hypothesis has intuitive appeal, due to the assumption that the elderly are less active. However, the fact that the muscles of speech, swallowing, and respiration¹³ undergo changes associated with aging argues against this hypothesis, as aged individuals speak, swallow, and breathe frequently within any given day. Nevertheless, we might consider attributing the differential changes in MHC alteration in the aged laryngeal adductory (LTA, LCA) versus abductor muscles (PCA) to the extent of neuromuscular activity, although greater or more sustained neuromuscular activity in the PCA muscle, a key component of the respiratory system, has not been documented. The differential manifestations in adductory versus abductor muscles discovered in this study are compelling and suggest that functions more critically tied to adduction, such as phonation and airway protection, may be more impaired in the elderly than those functions dependent upon abduction, such as respiration.

In conclusion, it appears from the alterations observed in MHC proteins that muscles of laryngeal adduction undergo a progressive change to a slower-contracting fiber type as a function of aging, which mirrors the changes observed after denervation. In this study, the ages of the old animals approximated the half-life of the particular strain of rat used. Therefore, these animals cannot be presumed to manifest severe age-related alterations commensurate with complete denervation. As such, further investigation in an older group of animals is warranted to determine whether these changes are progressive, and to determine the manner in which MHC protein changes affect physiology and function. Given that muscle fiber type transformations appear to be reversible with recovery from denervation injuries,²⁰ it would also be beneficial to determine whether the age-related decline in muscle contractile function in the larynx can be positively affected by particular interventions. In light of the serious and pervasive problem of swallowing disruption in the elderly, increased study of age-related changes in neuromuscular function in the head and neck appears crucial.

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EFFECTS OF PREDNISONE AND IBUPROFEN ON RADIO FREQUENCY VOLUME TISSUE REDUCTION IN A RABBIT MODEL

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This study investigates whether acute anti-inflammatory medications (prednisone and ibuprofen) alter muscle volumetric reduction following radio frequency tissue ablation (RFTA). We used a rabbit model to measure changes in leg muscle volume using serial magnetic resonance imaging in 3 groups: RFTA without medication (group 1), RFTA with prednisone and ibuprofen (group 2), and no RFTA or medication (group 3). The mean volumetric changes for groups 1 and 2 differed on days 1 and 7 (+0.5 cm³ versus -0.4 cm³, $p < .0001$; and -0.03 cm³ versus -0.7 cm³, $p < .05$), but not on day 28 (-0.8 cm³ versus -1.0 cm³, not significant). Group 3 had no change in volume. The volumetric reduction varied. Impaired volumetric reduction (<50% mean change) occurred in 30% of extremities and correlated to lower tissue impedance ($p < .04$). Combined steroidal and nonsteroidal anti-inflammatory medications did decrease acute edema, but not the final volumetric reduction, following RFTA. Volumetric changes are variable and may be altered by tissue impedance.

KEY WORDS — electrosurgery, muscle, obstructive sleep apnea, radio frequency, tissue ablation, wound healing.

Electricity has been used as a surgical tool for decades. Clark reported the first use of electricity for surgery in 1911, and Cushing and Bovie popularized its use in 1928.^{1,2} Most commonly, electricity has been used to cut and coagulate tissue. Radio frequency also has been used to ablate or volumetrically reduce tissues. Radio frequency tissue ablation (RFTA) has been described in multiple medical applications: liver metastasis, atrial fibrillation, benign prostatic hyperplasia, trigeminal nerve neuralgia, and snoring.³⁻¹² Volumetric tongue muscle reduction has been previously demonstrated in a porcine model and is now being evaluated as a less invasive alternative to traditional glossectomy for treatment of obstructive sleep apnea syndrome.^{13,14}

Radio frequency energy is an electromagnetic wave that has a frequency between 3.0×10^5 and 3.0×10^6 Hz.² This electromagnetic energy produces an alternating current in ionic mediums such as muscle. The current travels from the radio frequency generator through an electrode, ionic particles of the body, and the grounding pad and then back to the generator. The intrinsic resistance to the current in a tissue is responsible for the heat production and ablation of that tissue. The thermal energy from the RFTA causes cell death at 45°C, cell depolarization at 60°C, necrosis and edema at 70°C, and water vaporization at 100°C.² Typically, RFTA of the muscle is performed between 75°C and 85°C to cause cell necrosis and

edema. After the thermal injury, an inflammatory response occurs and necrotic debris and injured cells are removed over several hours to days during the catabolic phase of wound healing.¹⁵ Collagen and fibroblasts are deposited, organized, and contracted at the injured site during the anabolic phase of wound healing.¹⁶ Removal and destruction of muscle tissue, organization of collagen, and fibroblast contraction may contribute to muscle wound volumetric reduction.

Nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids, which are often used to control pain and decrease swelling after RFTA of the tongue base, decrease both inflammation and epithelial wound contraction.^{15,17-19} Potentially, NSAIDs and steroids may reduce muscle volumetric reduction (and treatment efficacy) by decreasing inflammation and wound contraction. To evaluate the effects of the acute anti-inflammatory medications, we developed an animal model to measure the combined effects of 2 anti-inflammatory medicines (NSAIDs and steroids) on the volumetric reduction of muscle after RFTA.

METHODS

Animals. Twenty-two mature male and female New Zealand rabbits weighing 5 to 6 pounds were used for the study. The animals were housed in separate cages and cared for according to the NIH *Guide for the Care and Use of Laboratory Animals* and Re-

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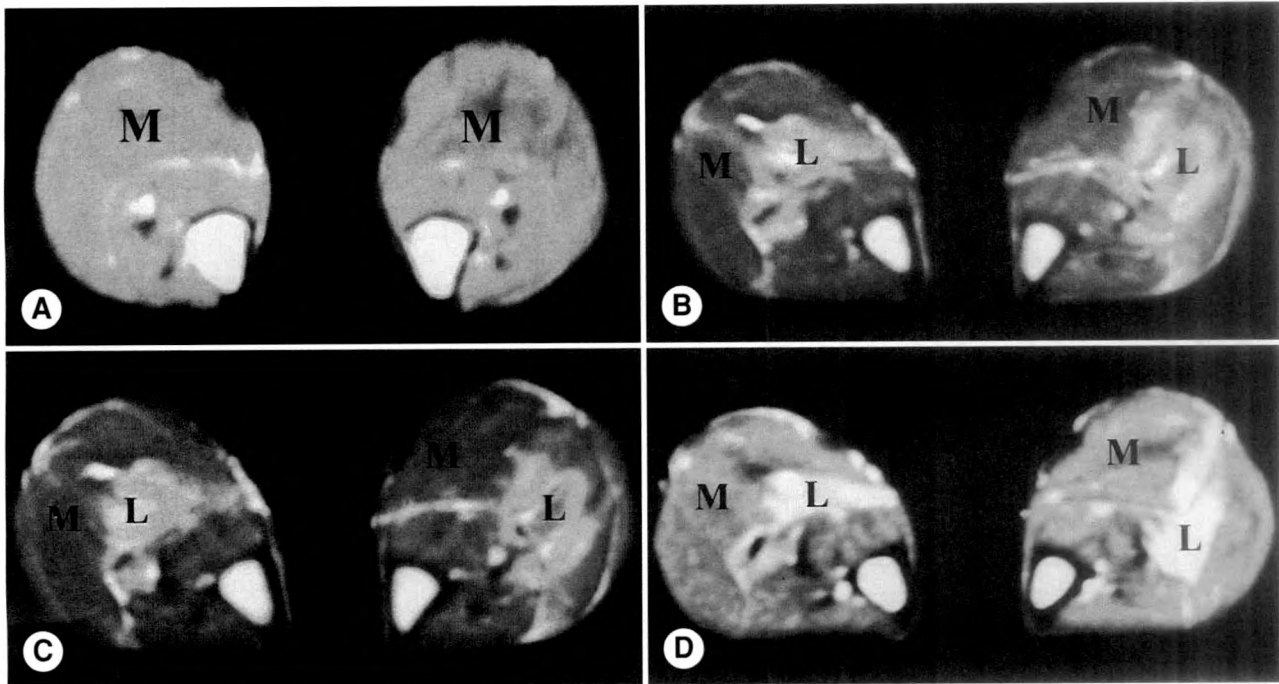


Fig 1. Axial fast spin density weighted magnetic resonance images of left and right legs. Images were taken on A) day 0, B) day 1, C) day 7, and D) day 28. Hyperintense mass (L) is site of ablation, and intermediate signal mass is muscle (M). Notice decreasing size of ablated lesion.

sponsible Use and Care of Animals in Research and Teaching. Xylazine hydrochloride (5 to 18 mg/kg) and ketamine hydrochloride (30 to 50 mg/kg) were used to anesthetize the rabbits during magnetic resonance imaging (MRI) and RFTA. Buprenorphine hydrochloride (0.05 to 0.1 mg/kg) was given for post-operative pain management. Prednisone (1 mg/kg per day) and ibuprofen (10 mg/kg every 4 hours) were given immediately before the operation and for 5 days after the operation.

Thermal Injury. A radio frequency generator (Somnus Medical Technologies, Inc, Sunnyvale, California) with custom-fabricated needle electrodes was used to ablate the muscle compartment of each leg. The radio frequency energy was delivered at 10 W for a total of 1,000 J to each leg muscle group (soleus and gastrocnemius muscle compartments). The microthermocoupler on the electrodes allowed the simultaneous recording of total energy delivered, test duration, resistance, and temperature. The electrode was positioned in the center of the muscle compartment as confirmed by palpation and subsequent MRI.

Volumetric Measurement. Volumetric measurements were performed with a 1.5-T MRI machine. Three-millimeter axial images were made through the leg from the knee to the ankle. Commercial software (Wildfire, GE Medical, Schenectady, New York) was used to measure the area of the leg muscle and then calculate the volume of the axial images based on the width of the slice. The volume of the muscle

from the knee to the ankle was calculated for each extremity.

Design. Forty-four extremities (22 rabbits) were used in the study. The rabbits were divided into 3 groups. Two active treatment groups received RFTA with no medicine (group 1, $n = 20$) and RFTA with prednisone and ibuprofen (group 2, $n = 20$). A third group (control) with no RFTA or medicine (group 3, $n = 4$) was included. For all groups, an initial (day 0) MRI scan was performed. For groups 1 and 2, RFTA was performed on the same day as the initial MRI. Imaging was performed on days 1, 7, and 28 (Fig 1). For the control group (group 3), MRI was performed 7 days after the initial MRI.

Statistical Analysis. A sample size of 20 was selected to identify a 25% volumetric reduction with a β error of .95. The results of the study were compared with an analysis of variance for multiple variables.

RESULTS

The weights of the rabbits did not change significantly during the time of the study. The 40 muscle compartments that received RFTA had an average initial volume of 18.1 cm³. The average volume had decreased by 0.9 cm³ (Table 1) by 28 days after the RFTA. The details of treatment time, energy, maximum temperature, and impedance for groups 1 and 2 are given in Table 2. The final volumetric reductions did not statistically differ between groups 1 and 2 ($p = .7$).

TABLE 1. VOLUME OF LEG MUSCLES FOR DAYS 0, 1, 7, AND 28

Day	Group 1	Group 2	Groups 1 and 2	Group 3
0	20.4 ± 3.8	15.8 ± 1.5	18.1 ± 3.7	14.6 ± 1.2
1	20.9 ± 3.8	15.4 ± 1.4	18.2 ± 4.0	
7	20.1 ± 3.4	15.1 ± 1.4	17.6 ± 3.6	14.6 ± 1.0
28	19.5 ± 3.3	14.8 ± 1.5	17.2 ± 3.5	

Data are in cubic centimeters (mean ± SD). Groups 1 and 2 had volume reduction. Group 3 did not have volume reduction.

Group 1 initially demonstrated an increased muscle volume, which decreased over the next 28 days (Fig 2). Group 2 had no immediate edema of the muscle compartments. The extremity volumes differed between groups 1 and 2 on days 1 and 7, but not on day 28. The control group, group 3, demonstrated no change in muscle volume (Table 1).

The volumetric reduction was not consistent in all the extremities that received RFTA. Impaired volumetric reduction was arbitrarily defined as a less than 50% decrease from the mean volumetric changes for groups 1 and 2. Thirty-five percent of group 1 and 25% of group 2 demonstrated impaired volume reduction, and the percentages of impaired volumetric reduction were not statistically different between the two groups. There was no statistical difference for total energy, maximum electrode temperature, treatment time, or site of the ablative lesion confirmed on MRI between the impaired and the nonimpaired groups. The impedances differed between the impaired (199 Ω) and nonimpaired (220 Ω) extremities ($p = .04$).

DISCUSSION

Steroids or NSAIDs are commonly given to patients after operation to prevent or reduce inflammation and swelling after RFTA. These medications are usually used for short durations following treatment. However, the use of these medications has the potential to adversely alter tissue volume reduction, since inflammation mediates both the anabolic and

TABLE 2. VALUES OF TOTAL ENERGY GIVEN, TIME OF TREATMENT, MAXIMUM ELECTRODE TEMPERATURE, AND AVERAGE IMPEDANCE

	Group 1	Group 2	Groups 1 and 2
Energy (J)	1,003.1 ± 1.7	1,002.8 ± 1.7	1,002.9 ± 1.7
Time (min)	2.7 ± 0.3	2.8 ± 0.4	2.7 ± 0.4
Maximum temperature (°C)	89.3 ± 1.3	87.3 ± 1.4	88.4 ± 1.7
Average impedance (Ω)	209.2 ± 27.6	218.6 ± 31.0	213.5 ± 30.0

Data are mean ± SD. All 3 groups had similar values in each category, and differences were not statistically significant.

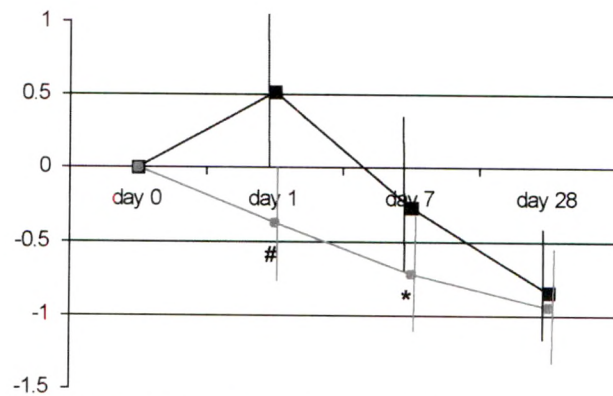


Fig 2. Change in initial volume (in cubic centimeters) on days 1, 7, and 28. Squares — group 1; circles — group 2; # — $p < .0001$; * — $p < .05$.

catabolic phases of wound healing. Since the effect of steroids or NSAIDs on the edema and volumetric reduction following RFTA of muscle is poorly understood, it was the goal of this study to identify whether the use of these medications may impair volumetric reduction of muscle. In order to maximize the effects of these medications, we used both steroids and NSAIDs simultaneously. Dosage of medicine was continued for 5 days to exceed the duration of acute tissue swelling.¹² Since we failed to observe a difference in volumetric change with both medications, it is unlikely that individual use of steroids and NSAIDs for short durations will impair volumetric reduction.

In group 1, which did not receive any anti-inflammatory medications, significant edema was observed immediately following treatment. Extremity volume increased an average of 0.52 cm³ on the first postoperative day. However, the group that received anti-inflammatory medications demonstrated an immediate decrease in volume. Since the reduction in extremity volume was immediate, this study indirectly suggests that the acute mechanism of volume reduction is unlikely to represent anabolic processes such as wound contracture (which occurs later in the healing process), and instead may represent destruction and removal of necrotic debris. Subsequent volumetric reduction may represent organization and contraction of the wound during the anabolic phase of wound healing. One weakness of this study is that it was performed in rabbit extremities that only partially replicate the human tongue. However, in contrast to other models, the rabbit model provided the advantage of repeated accurate measures of changes in a skeletal muscle compartment.

A novel finding in our study was the variability of volumetric reduction following RFTA. Thirty percent of all of the extremities that received RFTA had a volumetric reduction that was below 50% of the mean change in volume. This difference was correlated to

lowered impedance. A potential explanation for lowered impedance may be the presence of an electrical conduit, which could redirect the current away from the muscle of interest. This electrical conduit should have a lower impedance than muscle, eg, blood vessels.⁵ Although this conduit will not uniformly change the impedance of the muscle, it can change the average impedance the current travels through. Therefore, when a potential conduit is present, less energy will travel through the muscle, and a smaller ablative lesion will be created. However, since the difference in impedance between groups is small, it may not explain the difference in volume change. Other variables could have contributed to the impaired volumetric reduction. Variables that could alter the size of the ablative lesion and were controlled in the study were the maximum temperature of the tissue surrounding the electrode, the duration of RFTA, and

the free length of the radio frequency electrode.⁵ Variables that could alter the size of the ablative lesion and were not controlled in our study were the intrinsic resistance of the tissue being ablated and the uniform concentration of ionic particles surrounding the electrode.^{20,21} Identifying a cause for extremity differences in volume reduction remains speculative.

CONCLUSION

Prednisone and ibuprofen decrease the acute swelling after RFTA, but they do not affect the final volumetric reduction at 1 month. Furthermore, variations in volumetric reduction are observed that are associated with tissue impedance changes. Since consistent volume reduction is critical in clinical application of RFTA to obstructive sleep apnea syndrome, further evaluation of the techniques, tools, temperature, and ionic environment of RFTA is needed.

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COMPLICATIONS OF SUSPENSION LARYNGOSCOPY

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Although suspension laryngoscopy (SL) is routinely used in operative laryngology, no prospectively gathered data on the complications of this procedure have so far been available. We prospectively analyzed 339 consecutive procedures for intervention-related complications. The survey included preoperative dental status and assessment of postoperative dental, mucosal, and nerve injuries. Minor mucosal lesions were found in 75% of all patients. All healed spontaneously within a few days. Dental injuries occurred in 6.5% of all patients. These were more frequent in therapeutic laryngoscopy than in diagnostic procedures (6.8% versus 6.0%). Highly significant correlations were found between dental injury rate and preoperative dental disease ($p < .04$) and grade of periodontitis ($p < .001$). Temporary nerve lesions were observed in 13 patients (9 of the lingual nerve and 4 of the hypoglossal nerve). Although minor complications frequently occur during SL, it is a relatively safe procedure with a low risk of significant morbidity.

KEY WORDS — dental injury, perioperative complications, suspension laryngoscopy.

INTRODUCTION

Although suspension laryngoscopy (SL) is routinely used in diagnostic and operative laryngology, only limited data on the complications of this procedure have so far been available in the literature. Most studies assessed complications retrospectively without preoperative examination. Reported rates of minor complications such as dental injuries or minor bleeding range from 9.1%¹ to 31%² in patients who have undergone direct laryngoscopy alone or in conjunction with other endoscopic procedures. Major complications, defined as complications requiring hospitalization, were found in 19.5%.³ Hill et al⁴ reported a 1.2% incidence of airway complications requiring reintubation following SL. Atkins et al¹ had a 0.4% rate of emergency tracheotomy after direct laryngoscopy. Other life-threatening complications, such as pneumothorax, cardiovascular complications, and major bleeding, have also been reported.⁵⁻⁷

Dental injuries following laryngotracheal intubation for general anesthesia have been studied extensively, and a rate of 0.1% has been assessed.^{8,9} In contrast, there are no prospective data for dental injuries under the conditions of SL. This lack is somewhat surprising, as dental damage during that procedure is well known and dental protection is routinely recommended.

Injuries of the lingual nerve and hypoglossal nerve have been described in case reports¹⁰; however, general incidences have not been evaluated for SL.

The aim of this study was to assess the rate of and associated risk factors of intervention-related complications of SL in a prospective and well-defined series.

MATERIALS AND METHODS

We prospectively included all patients more than 18 years of age who were to undergo SL for any reason between August 18, 1997, and August 10, 1998, at the Department of Otorhinolaryngology–Head and Neck Surgery of the University of Cologne. The study population consisted of 339 consecutive patients. There were 252 men (74%) and 87 women (26%). The patients' ages ranged from 29 to 86 years, with an average of 59 years. Interventions were performed for the diagnosis or treatment of malignant tumors in 48% and noncancerous lesions (including control endoscopies following previous transoral surgery for laryngeal carcinomas) in 52%. The different treatment conditions are shown in Table 1. Suspension laryngoscopy was performed in 265 cases (78%) with the use of conventional cold instruments, mostly in a diagnostic approach, and in 74 cases (22%) in a laser surgery setting. All interventions were performed under general anesthesia. In 82% of cases, endotracheal

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TABLE 1. TREATMENT CONDITIONS

	Patients	
	No.	%
Interventions for malignancies	164	48
Tumor of larynx	69	21
Transglottic	12	4
Supraglottic	23	7
Glottic	32	9
Subglottic	2	1
Tumor of hypopharynx	33	10
Other carcinoma of upper aerodigestive tract	62	18
Interventions for benign lesions	175	52
Keratoses of larynx	24	7
Chronic laryngitis	12	4
Vocal cord granuloma	5	1
Vocal cord polyp	15	4
Vocal cord cyst	7	2
Laryngeal papillomatosis	4	1
Vocal cord palsy	16	5
Vocal cord edema	9	3
Epiglottitis	3	1
Botox injection	4	1
Laryngeal or subglottic stenosis	5	1
Zenker's diverticulum	2	1
Normal larynx (control laryngoscopy)	47	14
Others	22	6
Total	339	100

intubation was used, and in 18%, jet ventilation was chosen. A standard dental guard made from silicone was used for dental protection in all patients. All patients were examined before operation by one of the contributing head and neck surgeons (J.P.K., C.W., M.D., H.E.E.) and by a dentist (R.K.). The survey included preoperative dental examination in addition to complete clinical otorhinolaryngological examination. Within 24 hours of surgery, all patients were reexamined for intervention-related complications. Only injuries not related to resection or biopsy were counted as postoperative dental, mucosal, and nerve

injuries. Standardized forms for recording data were used. Patients with nerve injuries were followed up for 15 months. Factors associated with complication status were selected on cross-tabulations, which were analyzed by the use of a χ^2 test or *t*-test with the SPSS Base System, version 9.0 (SPSS, Chicago, Illinois). An initial significance level of $\alpha \leq 0.05$ was chosen.

RESULTS

Minor mucosal lesions were found in 75% of all patients after SL. In the 339 patients, a total of 518 mucosal injuries were counted. The distribution and types of mucosal lesions are shown in Table 2. Fifty-five percent of all lesions were erosions of the mucosa; all of these lesions were minor and healed spontaneously within a few days. However, there were also more severe injuries such as hematomas, which accounted for 29% of all lesions. Thirteen of the counted hematomas were located in the tongue, as shown in the Figure, A. Patients with this kind of complication had complaints for several weeks. Less frequent were lesions such as bleeding (7%), fissures (3%), and mucosal swelling (5%), as shown in Table 2.

Most of the evaluated mucosal lesions were found in the oral cavity: 172 of 339 patients (51%) had a mucosal lesion at that site. Overall, in the oral cavity, 265 lesions were counted, which constituted 51% of all mucosal lesions (Table 2). Most mucosal injuries of the oral cavity were located at the right or medial maxilla and accounted for 31% of all mucosal injuries. Injuries of the tongue (6%) were often hematomas (see Figure, A) or erosions. Some of those mucosal erosions, especially on the lateral part of the tongue, were painful and deep, as shown in the Figure, B.

The oropharynx was found to be the second most

TABLE 2. TYPES AND LOCATIONS OF ALL COUNTED MUCOSAL LESIONS (N = 518)

Location	Bleeding	Fissure	Erosion	Hematoma	Swelling	Total
Lips	4 (0.8%)	1 (0.2%)	62 (12%)	10 (2%)	15 (3%)	92 (18%)
Upper	1	0	17	7	2	27
Lower	3	1	45	3	13	65
Oral cavity	31 (6%)	11 (2%)	173 (33%)	44 (8%)	6 (1%)	265 (51%)
Right maxilla	17	4	60	4	0	85
Medial maxilla	14	6	57	0	0	77
Right tongue	0	1	12	13	4	30
All others	0	0	44	27	2	79
Oropharynx	0	6 (1%)	45 (9%)	98 (19%)	7 (1%)	156 (30%)
Right soft palate	0	1	23	70	7	101
Left soft palate	0	1	5	18	0	24
All others	0	4	17	10	0	31
Hypopharynx	0	0	5 (1%)	0	0	5 (1%)
Total	35 (7%)	18 (3%)	285 (55%)	152 (29%)	28 (5%)	518 (100%)



Typical complications after suspension laryngoscopy. **A)** Massive hematoma of tongue. **B)** Erosion of lateral part of tongue. **C)** Swelling of lower lip. **D)** Severe tooth loosening at front part of maxilla.

common site for mucosal lesions. The incidence of oropharyngeal lesions was 32% (110 of 339 patients). The most frequent injuries at that site were hematomas, which constituted 98 of 156 oropharyngeal lesions. They were predominantly located on the right side of the soft palate (101 of 165 oropharyngeal lesions).

Lip injuries were observed in 92 patients (27% of all patients and 18% of all counted mucosal lesions). In contrast to the oral cavity, in which most lesions were located at the upper jaw, most lip injuries were located on the lower lip (65 of 92 lesions, ie, 70.7%). Most of these injuries were superficial erosions. More severe swellings of the lower lip, as shown in the Figure, C, occurred in 3 patients. Mucosal injuries of the hypopharynx were rarely observed; only 5 of 518 lesions (1%) were located at that site.

To determine the influence of the operative setting, we compared mucosal injuries in diagnostic SL and therapeutic SL. For diagnostic SL and therapeutic SL, the rates of intervention-related mucosal lesions were 77% (205 of 265) and 68% (50 of 74), respectively. However, this difference was not significant ($p = .08$). The risk of mucosal lesions was higher for those patients who underwent SL under endotracheal intubation (76%) than for those who

underwent SL under jet ventilation (57%; $p < .05$).

Preoperative assessment of the dental status of all 339 patients revealed 65 patients (19%) as edentate and 9 (3%) as having no evidence of odontopathy. Of the remaining patients, 177 had periodontal disease only, 1 had carious teeth only, and 87 had both. Of the 264 patients with periodontal disease, 8% had gingivitis, 40% had superficial periodontitis, and 53% had deep periodontitis.

Overall, dental injuries were observed in 6.5% of all patients who underwent SL. In these 22 patients with intervention-related dental complications, 35 teeth were injured and 39 lesions were observed. The types of dental injuries and the preoperative dental status are shown in Table 3. The most frequently observed dental injuries were loosening of one or more teeth (43%). Most of the loosening was severe, and the teeth could not be preserved. The Figure, D, shows a typical severe tooth loosening that occurred after SL, located in the upper front part of the mouth. Fractures of the teeth and roots often required subsequent dental treatment and were observed in 24% of all dental injuries. Avulsions were found in 13% of the lesions. Other dental injuries (24%) included damage of fixed dental prostheses and fractures of dental fillings. Most dental injuries were located in the

TABLE 3. TYPES OF DENTAL INJURIES AND PREOPERATIVE PERIODONTAL STATUS

<i>Dental Injuries (n = 38)</i>	<i>Healthy</i>		<i>Gingivitis</i>		<i>Superficial Periodontitis</i>		<i>Deep Periodontitis</i>		<i>Total</i>	
	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Avulsion	0	0	0	0	0	0	5	13	5	13
Mild dental loosening	0	0	0	0	1	3	2	5	3	8
Severe dental loosening	0	0	0	0	0	0	13	34	13	34
Tooth or root fracture	0	0	1	3	0	0	8	21	9	24
Others	0	0	2	5	0	0	7	18	9	24
Total	0	0	3	8	1	3	35	92	38	100

upper front part of the mouth (60%), and there was a trend for the right side to be affected (Table 4). Ninety-two percent of all evaluated dental injuries occurred in patients who had been found before operation to have deep periodontal disease. The rate of dental injuries in this subgroup of patients was 13.3%. In contrast, no dental injury occurred in patients who had healthy teeth before SL. Significant correlations were therefore found between the dental injury rate and preoperative dental disease ($p < .04$) and grade of periodontitis ($p < .001$). Comparing the operative settings, we found dental complications to be more frequent in therapeutic laryngoscopy than in diagnostic procedures (6.8% versus 6.0%); however, the difference was not significant ($p = .91$). The rate of dental injuries was higher in patients who underwent SL under endotracheal intubation (6.8%) than in those in whom jet ventilation was performed (3.6%); however, the difference was not significant ($p = .51$).

Temporary nerve lesions were observed in 13 patients (3.8%). Nine patients had dysfunction of the lingual nerve, and 4 of the hypoglossal nerve. Patients with lingual nerve injury complained of tongue numbness, mostly localized to the right anterolateral aspect of the tongue. The duration of the complaints ranged from 1 to 60 weeks, with a median of 4 weeks (SD, 19.1 weeks). In all cases of hypoglossal nerve dysfunction, the right side was affected. The duration of the hypoglossal nerve dysfunction ranged from 6 to 16 weeks (median, 8 weeks).

In our series, no subject had airway complications that required reintubation or tracheotomy.

DISCUSSION

Suspension laryngoscopy is routinely used in diagnostic and operative laryngology. However, data on intervention-related complications are rare. The aim

of this study was to address this issue and to assess all detectable complications related to SL in a prospective and well-defined series.

We found no life-threatening complications and no intervention-related deaths. Therefore, we conclude that SL is a relatively safe procedure, and confirm similar data published earlier.¹¹

In contrast, we found mucosal lesions in 75% of our patients, which is a fairly high rate as compared to an earlier study.² In view of the number of included subjects and especially because of the prospective study design and our careful postoperative examination within 24 hours after endoscopy, we believe this estimate to reflect the true rate of mucosal lesions after SL. Because all of these lesions healed spontaneously without further treatment, they constitute no severe sequelae of endoscopy.

Furthermore, the knowledge of mechanisms that lead to mucosal injuries will help one to avoid them and may be helpful in learning the endoscopic technique of SL. Most lip lesions were located on the lower lip (Table 2 and Figure, C). These lesions often occur during insertion of the laryngoscope, if the lower lip is slipped over the lower teeth and becomes trapped between the teeth and the laryngoscope. In the same way, the tongue can be trapped between the frontal or lateral teeth and the laryngoscope and incur lesions such as that shown in the Figure, A. Such tongue hematomas lead to complaints that last for several weeks and should be avoided. Furthermore, mucosal injury seems to be related to the duration of intervention, as the rate was decreased in procedures performed under jet ventilation, which normally have a shorter duration. Interestingly, most mucosal, dental, and nerve lesions were located on the right side of the patient, reflecting the right-handedness of most surgeons.

TABLE 4. NUMBERS AND LOCATIONS (FDI WORLD DENTAL FEDERATION SCHEMA) OF INJURED TEETH

No. of injured teeth		1		2		4		6		6		1		1			
Maxilla	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	
Mandible	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	
No. of injured teeth		4		1		2		4		1		1		1			

For laryngotracheal intubations, dental injuries have been studied extensively, and most authors found rates of about 0.1%.^{8,9,12} In contrast, making an immediate postoperative assessment, Chen et al¹³ found a rate of 12% for minimal dental injuries after general anesthesia. However, for SL, no prospectively gathered data have been available so far. The dental injury rate has been retrospectively assessed to be 1%.¹⁴ In contrast, we found an overall dental injury rate of 6.5%. Therefore, SL may have a significantly higher risk for dental complications than laryngotracheal intubation. However, this conclusion may be limited by differences in the study populations concerning dental status and age.

In our series, subjects with deep periodontal disease had a highly increased risk of dental injury during SL ($p < .001$; odds ratio, 9.86; 95% confidence interval, 2.86 to 34.01). Therefore, in these patients, if the teeth are in a condition to be preserved, special dental guards may be useful.^{11,15-17} Otherwise, if periodontitis is so severe that the teeth have to be removed, it may be better to remove them before operation or during endoscopy with the patient's consent. On the other hand, it should be pointed out that in our study, dental injuries in subjects with healthy teeth were not observed.

Dental protection during SL is routinely used for the upper jaw because of the assumption that most pressure is applied to the upper front teeth. Interest-

ingly, we found 40% of all dental injuries to occur in the lower jaw, and surgeons may not be sufficiently aware of this specific risk. The rate of dental injury was increased in therapeutic interventions, such as endoscopic oncological procedures. In such interventions, the need for repeated readjustment of the endoscope is likely to contribute to the elevated rate of complications.

Nerve injuries after SL are known from several case reports. We first report here the incidence of this complication after SL. We found a 2.6% rate for lingual nerve injury with a median duration of 4 weeks and a 1.1% rate for hypoglossal nerve injury with a median duration of 8 weeks. Although these were temporary lesions, we recommend that patients should be informed about these risks before operation.

CONCLUSION

Suspension laryngoscopy carries a higher risk for minor mucosal lesions, tooth injuries, and temporary lingual and hypoglossal nerve injuries than was previously anticipated. In contrast, the rate of life-threatening complications was lower than that reported in the literature.

We conclude that SL is a relatively safe procedure. The severity of the complications compares favorably to that of complications of open surgery and qualifies SL as a routine surgical approach to a wide variety of laryngeal disorders.

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SOFT TISSUE RESPONSE OF THE RABBIT LARYNX TO GORE-TEX IMPLANTS

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The use of Gore-Tex (expanded polytetrafluoroethylene) for medialization laryngoplasty has recently been described. Clinically, this laryngeal implant has performed well, but no animal studies evaluating the use of this implant in the larynx have been published. This study examines the gross and histologic response of the rabbit larynx to the Gore-Tex implant. Five rabbits underwent bilateral medialization with the Gore Thyroplasty Device. The vocal folds were left innervated and mobile. After 6 months, the rabbits were painlessly sacrificed and the implants were evaluated by gross inspection and histologic examination. No gross changes to the glottis occurred, and the implants were easily removed with gentle traction. A modest inflammatory response with a thin fibrous capsule was present around the implant. Foreign body giant cells were observed at the interface of the implant with the tissue. No migration or extrusion of the implant was observed. These results suggest that Gore-Tex is biocompatible in the rabbit larynx.

KEY WORDS — ePTFE, expanded polytetrafluoroethylene, Gore-Tex, medialization laryngoplasty, vocal cord, vocal fold bowing, vocal fold paralysis.

INTRODUCTION

Medialization laryngoplasty is a common procedure used to restore glottic competence. This procedure was popularized by Isshiki and initially used in patients with unilateral vocal fold paralysis. In recent years, the indications for this procedure have expanded to include most forms of glottic incompetence, including the use of bilateral medialization in mobile vocal folds for vocal fold bowing and atrophy.

Although medialization is now widely accepted, the choice of implant material remains controversial. Injection of autologous tissue such as fat or fascia is well described and has the benefit of medializing the affected cord while provoking minimal tissue response.¹ Similarly, collagen injection is being used with success.^{2,3} Unfortunately, injectable biological implants are often temporary, and serial injections may be required.

A myriad of relatively permanent synthetic materials have also been used to restore glottic competence. Injection of Teflon paste (polytetrafluoroethylene) is well described in the literature.^{4,5} Because of problems associated with this implant, its use has fallen into disfavor. Flint et al⁶ evaluated the soft tissue response of the rabbit larynx to this material, and

problems with the medialization were evident. Teflon provoked a granulomatous response in many subjects and was prone to migration from the site of implantation, as well as extrusion into the airway.⁶ Also, removal of the material has proven problematic because of the surrounding tissue response.⁷

Materials placed transcutaneously (as opposed to materials injected endoscopically) also have been evaluated in the literature.^{8,9} Simpson et al¹⁰ described the histologic response of the dog larynx to silicone elastomer (Dow Corning) implants in mobile vocal cords and found that the implant was well tolerated. Minimal chronic inflammation and a thin fibrous capsule were observed around the implant.¹⁰ Flint et al⁶ evaluated silicone elastomer and hydroxylapatite implants in paralyzed vocal cords in the rabbit model cited above. Findings similar to those seen in the study by Simpson et al were observed for both implants.

Recently, the use of the expanded polytetrafluoroethylene implant (ePTFE or Gore-Tex) for medialization was reported.¹¹ Gore-Tex has been used extensively in vascular and cardiac surgery with minimal complications, and its use in soft tissue augmentation has increased in recent years. The majority of studies in the literature demonstrate that the implant is a relatively safe and relatively inert means to aug-

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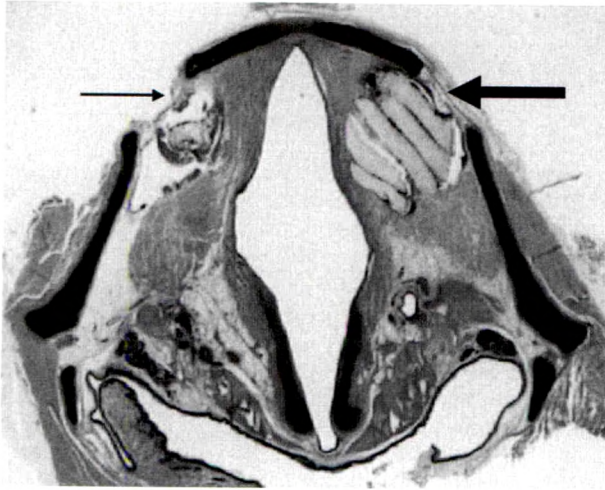


Fig 1. Low-power H & E stain of rabbit larynx demonstrates right thyroid cartilage window with Gore-Tex implant in place (broad arrow), as well as left thyroid cartilage window with space remaining after removal of implant (thin arrow).

ment soft tissue.¹²⁻¹⁴ Several reports, however, have described extrusion and granuloma formation.¹⁵⁻¹⁷ Concerns regarding its safety have been advanced, particularly in light of its being similar in composition to Teflon. Concern has also arisen over the use of this implant in dynamic areas (such as the larynx) in which movement of overlying tissue might promote extrusion.^{5,18}

Currently, no animal studies have evaluated the Gore Thyroplasty Device used in the standard method of insertion through a thyroid cartilage window. Tamplenizza et al¹⁹ described placement of tubular Gore-Tex implants through a trocar in the pig larynx. Their method of insertion and the structure of their implant are experimental and are not used in humans currently. Histologic findings on this method of insertion are not yet available. The goal of this study was to evaluate the gross and histologic responses of mobile vocal folds to the Gore Thyroplasty Device in a rabbit model. The specific interests of this study included 1) Does the implant produce grossly observable changes in the surrounding soft tissue? 2) Is the implant easily removable? 3) Does the implant extrude or migrate from its site of implantation? 4) Does the implant provoke histologically observable changes in the surrounding tissue? 5) Does surrounding tissue grow into the implant?

MATERIALS AND METHODS

Five New Zealand White rabbits underwent general anesthesia. The neck was shaved and the surgical site prepared with iodine solution. Under sterile conditions, a horizontal incision was made over the thyroid cartilage. The strap muscles were divided in the midline and retracted laterally, exposing the thy-

roid perichondrium. A 2-mm skin punch was used to create a window through the thyroid cartilage. The window was 3 mm lateral to the midline, and a 2-mm strut of cartilage was left inferiorly. Previous surgical dissection had demonstrated that this was the position of the midportion of the vocal fold. The inner perichondrium was preserved. A strip of Gore-Tex (Gore Thyroplasty Device, W. L. Gore and Associates) measuring 25 mm in length, 2 mm in width, and 0.6 mm in thickness was folded 5 times and inserted into the subperichondrial space through the cartilage window and secured with a 6-0 Prolene suture to the inferior cartilaginous strut. Placement of the implant at the glottic level was confirmed by direct laryngoscopy. The implant was considered to be in place if the vocal fold was medialized without disruption of the surrounding glottic tissue. The procedure was then repeated on the opposite side. The strap muscles were reapproximated in the midline with 3-0 Vicryl. The skin was closed with 3-0 Vicryl and 4-0 chromic sutures. The rabbits were given a 0.5-mL subcutaneous injection of Baytril (Bayer, Shawnee Mission, Kansas) each day for 5 days after operation.

At 6 months after implantation, the rabbits were painlessly sacrificed with an intravenous injection of pentobarbital sodium (150 mg/kg). The larynges were harvested, and the vocal cords were visualized to ensure that no visible abnormalities were present in the airway. From each rabbit, 1 implant was selected for gross evaluation. The suture securing the implant to the cartilaginous strut was cut, and the implant was grasped with forceps and removed. Ease of removal was graded by dividing the specimens into 2 groups according to whether 1) the implant was removable with gentle traction or 2) dissection of the implant with instruments was required for removal. Any apparent damage to the surrounding tissue following removal was noted. Histologic evaluation of this specimen was performed to check for residual implant material. Additionally, the removed implant was sent for microscopic evaluation.

Each larynx was then sent (with the contralateral implant in place) for histologic evaluation (Fig 1). The position of the implant was described, and any migration of the implant was measured relative to the glottic level. The presence of tissue growth into the implant was measured on the basis of depth of penetration. Granuloma formation and other inflammatory changes were noted, and if they were present, the maximum diameter of the inflammatory process was measured. The number and type of inflammatory cells were recorded. Any fibrotic changes in the surrounding tissue were also noted, and the extent of the fibrosis was measured.

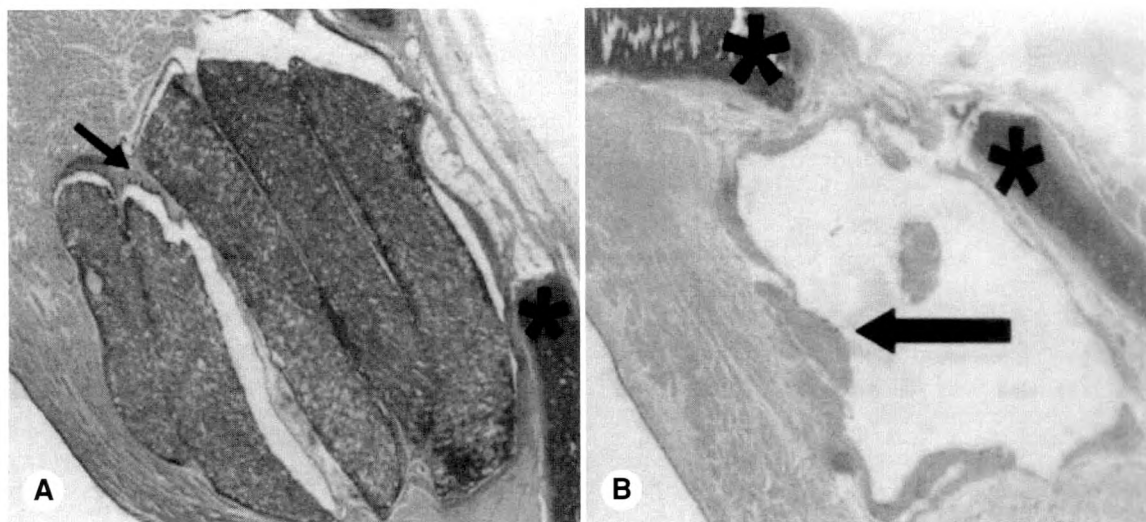


Fig 2. Medium-power H & E stains. Asterisks mark edges of thyroid cartilage window. **A)** Implant with fibrous ingrowth between folds (arrow). **B)** Residual fibrous capsule (arrow) that remains after implant is removed.

RESULTS

All animals underwent successful medialization, and all animals lived to the completion of the study. At the time of harvest, no rabbit had any visible airway abnormality. The area of maximal medialization was difficult to assess because of the small size of the implant, but no medialization of supraglottic or subglottic structures was observed in any animal.

In the gross evaluation, 3 left-sided implants and 2 right-sided implants were removed. All of the implants were easily identified by the retention suture, which remained in place, fastened to the cartilage strut. The implant was removable with gentle traction in all cases, and no damage to surrounding tissue was apparent with the implant's removal. No gross changes to the surrounding tissue were observed.

The microscopic evaluation of the removed implants showed isolated foreign body giant cells and

scant fibroblasts adherent to the ePTFE. No measurable growth of tissue into the implant was observed.

Microscopic analysis of each larynx (with 1 implant in place and 1 implant previously removed during the gross evaluation) revealed a slight inflammatory reaction with foreign body giant cells present at the interface of the implant and the tissue. A thin fibrous capsule measuring between 0.01 and 0.1 mm surrounded the implant. The fibrous capsule extended between the folds of the implant, but no unrolling of the implant occurred, and no infiltration into the implant itself was present. All implants were at the glottic level and were appropriately positioned. No histologic changes in the thyroarytenoid muscle or in the overlying epithelium of the vocal cord were observed. Scant residual implant material was seen in the capsule of some sites from which the implant had been removed (Figs 2 and 3).

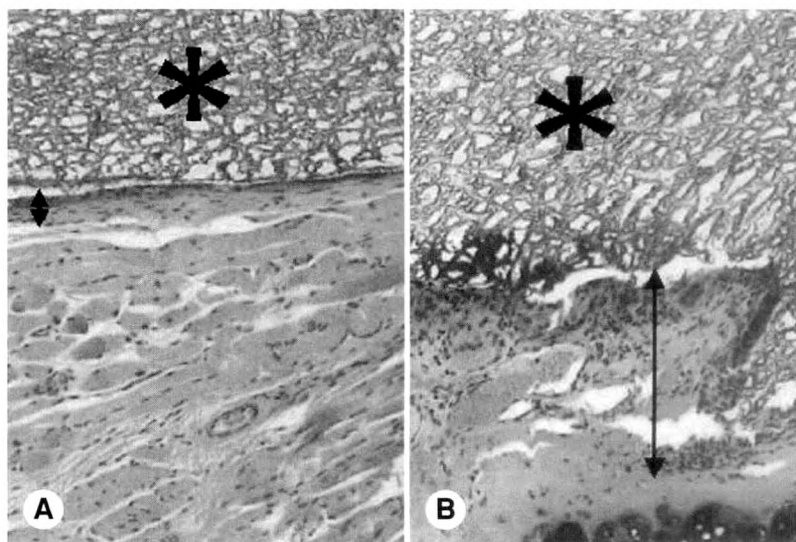


Fig 3. High-power H & E stains. Asterisks mark implant. Arrows demonstrate **A)** minimal (0.01 mm) and **B)** maximal (0.1 mm) fibrous and inflammatory responses to Gore-Tex implant.

DISCUSSION

The type of implant used for permanent medialization of the vocal fold remains a controversial subject. The ideal permanent implant should be completely inert in tissue and nonbiodegradable. The implant should be well anchored to decrease the risk of extrusion, and it should be compliant so as to mimic normal tissue. It must also be firm, to maintain its shape and medialize the cord appropriately. Finally, the implant should be easily moldable and readily positioned. Currently, no ideal implant has been identified, but the strengths and weaknesses of available materials can be compared.

Proponents of the Gore-Tex implant point out that it has several advantages over other materials used in medialization.^{11,20-22} The Gore-Tex implant is soft and easy to handle. As compared with use of silicone, the Gore-Tex medialization procedure is faster and less technically demanding, because the implant does not need to be carved. During the surgery, the implant can be shaped after insertion into the patient, whereas other implants require removal if the implant must be modified. Because this is a framework type of medialization (as opposed to an injection), this procedure is easily combined with other interventions designed to restore glottic competence, such as arytenoid adduction. Also, proponents claim that the implant is easy to remove, should the procedure need to be reversed.^{20,22}

Several reports in the literature describe this implant in clinical use for medialization laryngoplasty. McCulloch et al^{11,20} evaluated 65 patients in 2 studies. Two of the patients had postoperative dyspnea, and 1 of these required tracheostomy. Three patients required revision surgery for implant modification. In 2 of the patients, the implant was easily removed without complications at 2 and 6 months after implantation. In the third patient, no information was given regarding the revision procedure.

Zeitels²¹ reported more than 60 cases of Gore-Tex medialization with "minimal complications." Giovanni et al²² reported on 11 patients. One of these required removal of the implant 5 months after placement because of exuberant granulation tissue surrounding the implant. The implant was removed endoscopically without difficulty. None of these studies reported problems with extrusion or migration of the implant.

Gore-Tex has been used extensively in cardiovascular surgery, with an excellent record of biocompatibility. It has also been reported fairly extensively in the facial plastics literature as being used to augment soft tissue. The biocompatibility of the implant in facial plastic surgery is excellent. Godin et al²³ re-

viewed more than 900 cases and found an overall complication rate of 2.2%. The vast majority of these complications were infections. No reports of extrusion of the implant have been published in any of the retrospective series in which the implant has been used to augment facial soft tissue.^{17,23-32} Two isolated case reports have appeared describing extrusion of Gore-Tex implants used to augment the nasal dorsum.^{15,33} When these 2 case reports are added to the body of the literature, the number of facial plastic implants reaches 1,125, with an extrusion rate of 0.2%.

The long-term behavior of Gore-Tex in the larynx is unknown, and the glottis presents several challenges to the implant that are not seen in other areas in which the material has been studied: this implant lies just deep to the vocalis muscle and is subjected to the vibratory and dynamic motions of the glottis. The mere possibility of extrusion or a soft tissue reaction raises concern because of the risk of airway compromise and permanent vocal disability.

In this evaluation of the Gore-Tex implant, we chose to use bilateral implants in mobile vocal cords. Performing bilateral implantation allowed us to evaluate the ease of removal of an implant and to view an implant *in situ* in the same test animal. By allowing the vocal cords to remain mobile, we could evaluate the implant in a dynamic situation. We wanted to evaluate the long-term effects of this implant, and a period of 6 months was chosen. Granulomatous reactions to some implant materials can evolve over several years. However, there are 3 animal studies that suggest a mature response to the ePTFE implant within 6 months.^{12,13,24}

Reports of extrusion of Gore-Tex implants are found in animal models. Using a porcine model, Maas et al¹² noted a tendency of the ePTFE implants to migrate and extrude through the skin. In their study, ePTFE placed subcutaneously extruded at a rate of 4%. All of the extrusions occurred within the first 6 months. They concluded that the limited fibrous growth into the surface of the material failed to provide enough stability, particularly when the implants were in mobile tissue. Hollow cylinders of Gore-Tex, which allowed fibrous growth into the lumen, appeared more stable and were less likely to extrude. In his rabbit model, Neel¹³ noted that the limited fibrous growth into the standard Gore-Tex implant seemed to leave it insecurely embedded in the tissue. The use of Gore-Tex with a larger pore size (allowing fibrous ingrowth) stabilized the implant without sacrificing easy removability. The implants were placed underneath the thin skin of the rabbit pinna, and despite concerns regarding stability, no extru-

sions of any of the Gore-Tex implants occurred.

In our investigation, no extrusion of implants occurred. The implants were easy to remove, although a moderate amount of traction was required, suggesting attachment to the surrounding tissue. All implants remained at the glottic level with no evidence of migration. The small size of the implant hindered more precise measurements. High-resolution computed tomographic scanning could possibly provide insight into implant migration not detected by our methods.

Histologic evaluation of the Gore-Tex implant has shown it as one of the most biologically inert implants available. No reports of granuloma formation from an implant in an animal model have appeared, but 6 cases of an exuberant soft tissue response that required implant removal were identified in the facial plastics and otolaryngology literature.^{16,17,21,32} The only case involving the larynx was mentioned previously; granulation tissue resulted in the removal of the laryngeal implant.²¹ The second case involved ePTFE thread used in lip augmentation and showed a foreign body reaction consistent with granuloma formation.¹⁶ No histologic description is provided in the remainder of the cases.

In our histologic examination, we found foreign

body giant cells and a thin rim of fibrous tissue surrounding the implant. The fibrous capsule invaginated between the folds of the implant, but did not appear to grow into the implant itself. The fact that the implant remained in place through the sectioning process shows that the implant was secure in the soft tissue. These findings agree with the consensus of the literature in which the histology of the implant has been studied in animal models in nonlaryngeal sites.¹²⁻¹⁴ The soft tissue response to the Gore-Tex laryngeal implant is similar to the response seen with silicone elastomer medialization.^{6,10}

CONCLUSION

The results of this study suggest that the Gore-Tex implants are biocompatible in the rabbit larynx. These implants remain sufficiently secure in the soft tissue to prevent migration and extrusion, but are removed easily if the procedure needs to be reversed. The histologic findings in this animal model are similar to those of other popular implants such as silicone elastomer as described in the canine model¹⁰ and hydroxylapatite as described in the rabbit model.⁶ The slight inflammatory response and the thin layer of fibrosis suggest that this implant is well tolerated immunologically.

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ECONOMIC BENEFIT OF TONSILLECTOMY IN ADULTS WITH CHRONIC TONSILLITIS

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To determine the economic impact of adult chronic tonsillitis and the economic improvement from adult tonsillectomy, we studied patients who underwent adult tonsillectomy for chronic tonsillitis with the Glasgow Benefit Inventory and a questionnaire for disease severity parameters before and after tonsillectomy. The economic impact of chronic tonsillitis and adult tonsillectomy were computed with a break-even time analysis model. Eighty-three adult patients (average age, 27.3 years) completed the study with an average duration of follow-up of 37.7 months. The patients exhibited a mean improvement in quality of life of $+27.54 \pm 4.63$ after tonsillectomy according to the Glasgow Benefit Inventory. In the 12 months following the procedure, tonsillectomy resulted in yearly mean decreases in number of weeks on antibiotics by 5.9 weeks, number of workdays missed because of tonsillitis by 8.7 days, and physician visits for tonsillitis by 5.3 visits. In considering the medical costs of tonsillectomy only, the break-even point was found to be 12.7 years, whereas considering the overall economic impact of tonsillectomy resulted in a break-even point of 2.3 years after the procedure. We conclude that tonsillectomy results in significant improvement in quality of life, decreases health-care utilization, and diminishes the economic burden of chronic tonsillitis in the adult patient population.

KEY WORDS — chronic tonsillitis, economic impact, tonsillectomy.

INTRODUCTION

Many surgical interventions in otolaryngology attempt to cure or relieve symptoms related to chronic or persistent infectious processes within the head and neck. Examples include endoscopic sinus surgery for chronic rhinosinusitis, tympanomastoidectomy for chronic otitis media, and tonsillectomy for chronic tonsillitis or pharyngitis. Although some of these conditions may be successfully managed with medical therapy, when the disease process becomes refractory, surgery is then considered. Despite the fact that surgery is often considered only after exhausting all medical therapeutic options, so that selection bias brings to surgery only the more refractory cases, these surgical interventions have been found to be clinically effective in the management of these chronic infectious disorders.^{1,2}

In the current era of cost containment and diminishing health-care allocation, evidence of clinical effectiveness is often not enough to justify specific therapeutic modalities. Therapies, especially interventional ones, must also be found to be cost-effective in the management of disease in order to be adopted by caregivers, institutions, and health-care plans. Often, interventional therapeutic modalities carry with them greater initial health-care expenditures, which make them appear less attractive from an economic standpoint.

Although tonsillectomy has been shown to be ef-

fective in the management of pediatric sleep-disordered breathing and chronic tonsillitis, less is known about the clinical effectiveness of tonsillectomy in the adult patient population for the indication of chronic tonsillitis. Mui et al³ have shown that tonsillectomy significantly reduces the need for antibiotic therapy and physician visits for adult chronic tonsillitis. The goal of the present study was to determine the economic impact of chronic tonsillitis in the adult population, and to determine the cost savings associated with tonsillectomy in this population.

Break-even time analysis is a method of determining when the introduction of a new product or service will generate enough revenue to recover the up-front expenses generated by the new product or service. In 1998, Gliklich and Metson⁴ introduced the concept of break-even time analysis to the otolaryngology literature by studying the economic impact of endoscopic sinus surgery on the management of chronic rhinosinusitis. In their important work, they proposed a method of break-even time analysis that can be readily applied to otolaryngological interventions. In terms of health-care intervention, break-even time analysis involves the determination of the point in time at which the up-front cost of the intervention (for example, a surgical procedure) is recovered by the decreased health-care cost (for example, decreased medication use or decreased physician visits) of managing the disease condition in the subsequent years after the intervention.^{5,6} Given that illness often im-

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parts a cost to society in terms of lost productivity in addition to health-care costs, a more global concept of break-even time analysis could include the overall economic impact of an intervention and recovered costs after therapy.⁴ Break-even time analysis was chosen in this study to model the economic impact and influence of tonsillectomy on chronic tonsillitis in the adult patient population.

METHODS

This study was approved by our institutional human studies committee. For the time period 1994 to 1999, 293 adult patients who underwent tonsillectomy for a diagnosis of chronic tonsillitis were identified from a procedural database from a large academic otolaryngology practice. All patients met American Academy of Otolaryngology clinical practice guidelines for consideration for adult tonsillectomy. Patients with a follow-up of less than 1 year were excluded. Patients who underwent tonsillectomy for treatment of obstructive sleep apnea syndrome or for potential malignancy were also excluded, as were patients who simultaneously underwent adenoidectomy.

This cohort was evaluated by chart review and mail survey. The components of the survey included patient-reported data for disease severity parameters. These disease parameters included the number of weeks in the 12 months prior to tonsillectomy during which the patient was taking antibiotics specifically for tonsillitis, the number of days of work missed because of sore throats in the 12 months prior to tonsillectomy, and the number of physician visits specifically for sore throats during the same 12 months prior to tonsillectomy. Analogous data were collected for the 12 months after tonsillectomy (excluding the immediate postoperative period). Each patient also completed the Glasgow Benefit Inventory (GBI), which was modified to measure the change in health status and quality of life due to the tonsillectomy intervention.⁷ The GBI scores were scaled in standard fashion to range from -100 to +100, with positive scores implying an improvement in quality of life due to tonsillectomy. Statistical analysis was conducted with SPSS version 10.0 (SPSS Inc, Chicago, Illinois). Descriptive statistics were calculated for patient demographics and mean follow-up. Statistical comparison between disease parameters before and after tonsillectomy was conducted with Student's paired *t*-test.

Economic cost analysis was then conducted according to previously described methods, similar to the method adapted by Gliklich and Metson.^{4,6} From the patient-reported data regarding number of phy-

sician visits and number of weeks on antibiotics during the 12-month period prior to tonsillectomy, the medical cost of chronic tonsillitis was assessed. The economic cost of the physician visits was determined by multiplying the mean number of visits by the average Medicare reimbursement for E/M (evaluation and management) visit codes 99212 and 99213. Antibiotic cost was determined by multiplying the mean number of days on antibiotics by the average wholesale prices for penicillin, amoxicillin, and erythromycin treatments per day based on 1999 data. The non-health-care economic impact of chronic tonsillitis was assessed by multiplying the mean number of workdays missed because of chronic tonsillitis exacerbations by the national gross domestic product (GDP) per capita per day. All economic values were assigned with 1999 figures. Similar calculations for the costs due to physician visits, antibiotic therapy, and workdays missed were computed for the 12-month period after tonsillectomy for each patient.

These data were then entered into a computerized spreadsheet. The cost of an adult tonsillectomy was assessed with 1999 cost data. The medical costs for adult tonsillectomy were determined by summing the surgeon's reimbursement, anesthesiologist's reimbursement, and hospital facility reimbursement for an uncomplicated outpatient tonsillectomy with the cost of 10 days' postoperative therapy with oxycodone hydrochloride and standard oral antibiotic therapy. The reimbursements were determined by averaging the 1999 figures for 3 separate third-party payers in our geographic area. The postoperative antibiotic costs were determined from the average wholesale price for amoxicillin in 1999. The overall economic cost of adult tonsillectomy was assessed by adding the economic cost of 5 days of work absence from tonsillectomy to the above medical costs of tonsillectomy. The economic cost of 5 days of work absence was determined from the GDP per capita per day based on 1999 economic figures.

Break-even time analysis was then conducted by projecting the cost savings due to tonsillectomy over several years after tonsillectomy. Medical costs were adjusted for inflation with the medical subset of the consumer price index (CPI-M). The CPI-M was assumed to be steady at 1999 levels. The GDP cost was adjusted over subsequent years with the 1999 GDP growth rate, also assumed to be constant over subsequent years. The break-even point at which the upfront cost of tonsillectomy was recovered by cost savings from decreases in medication requirements, physician visits, and workdays missed was computed. A medical cost break-even point was computed, corresponding to cost savings related to medication use

TABLE 1. QUALITY-OF-LIFE IMPACT OF ADULT TONSILLECTOMY AS MEASURED BY GLASGOW BENEFIT INVENTORY

Score	Mean	±95% CI	p*
Total score	+27.54	4.63	<.001
General subscore	+35.74	5.33	<.001
Social subscore	+15.46	5.55	<.001
Physical health subscore	+6.83	4.83	.003

CI — confidence interval.
*Student's *t*-test for population.

and physician visits, and an overall break-even point was computed, including medical cost savings and cost savings due to decreased illness-related work absences. Sensitivity analysis was conducted in order to test the assumptions of the model and determine factors that influenced the break-even point.

RESULTS

A total of 83 adult patients completed and returned their surveys (response rate, 30.3%). The mean patient age was 27.3 years (SD, 8.5 years), and the average follow-up duration after tonsillectomy was 37.7 months. The mean scores on the GBI for the total score and the subscores are presented in Table 1. These data provide an overall estimate for the quality-of-life impact of adult tonsillectomy in this population. Table 2 presents data for the mean number of weeks on antibiotics, mean number of physician visits, and mean number of workdays missed for the 12 months before and after tonsillectomy. The decrease in each of these disease severity parameters was statistically significant, as indicated in Table 2.

Determination of Reference Cost Data. The mean cost of a physician visit for tonsillitis was determined to be \$36.97. The mean cost of therapy with 10 days of antibiotics for tonsillitis was determined to be \$7.93. The US GDP per capita per day was \$129.89, according to 1999 government economic figures.

Determination of Cost of Treatment. The mean cost of medical treatment for chronic tonsillitis in the 12 months prior to tonsillectomy was \$242.89 per patient. The mean cost related to work absence due to chronic tonsillitis during the 12 months prior to tonsillectomy was \$1,128.88 per patient. The mean cost of medical treatment specifically for chronic tonsillitis in the 12 months after tonsillectomy was reduced to \$14.17, and the mean cost related to work absence was reduced to \$81.78 per patient. This calculation results in a net cost savings of \$228.72/y for medical costs, \$1,047.10/y for work-related economic costs, and a total economic cost savings of \$1,275.82/y after tonsillectomy.

The medical cost of adult tonsillectomy was deter-

TABLE 2. IMPACT OF CHRONIC TONSILLITIS BEFORE AND AFTER TONSILLECTOMY

Disease Severity Measure	12 Months Before Tonsillectomy		12 Months After Tonsillectomy		Mean Net Change	p*
	Mean	SD	Mean	SD		
No. of weeks on antibiotics	6.5	6.4	0.6	0.9	-5.9	<.001
No. of days of work missed	9.2	11.8	0.6	2.0	-8.7	<.001
No. of physician visits	5.7	5.3	0.3	0.8	-5.3	<.001

*Student's paired *t*-test.

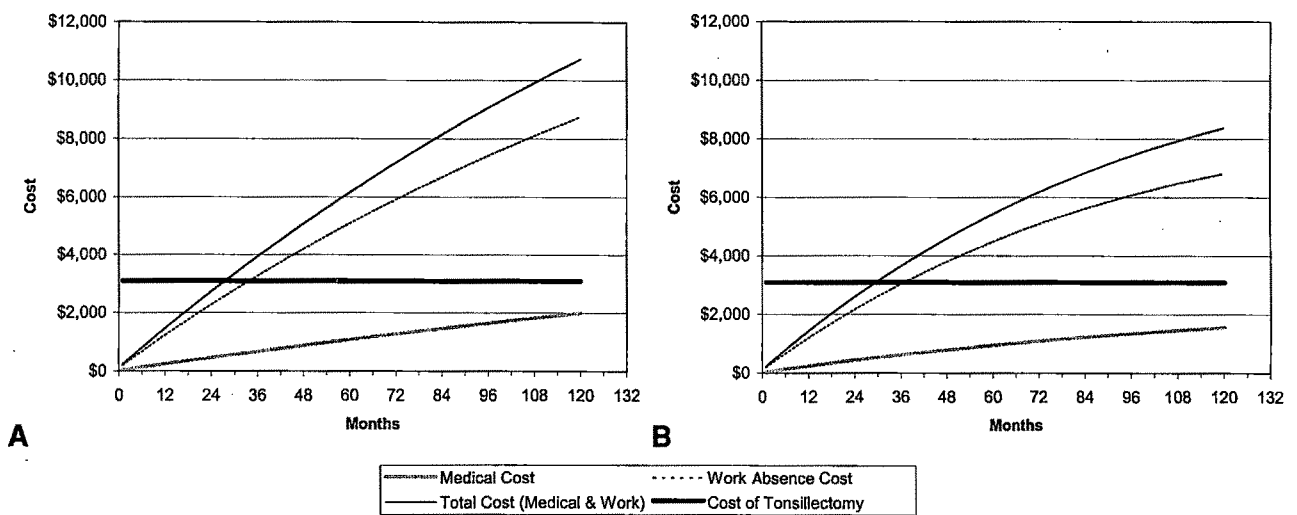
mined to be \$2,444.91, including the surgeon's reimbursement, anesthesiologist's reimbursement, hospital reimbursement, and postoperative medication. The lost-work economic impact for 5 business days after tonsillectomy was determined to be \$649.43. This calculation results in a net total cost for tonsillectomy of \$3,094.34.

Description of Break-Even Time Analysis. Break-even time analysis was conducted on the basis of the above cost factors and inflation indices. The Figure, A, graphically displays the basic break-even time analysis for adult tonsillectomy. The medical costs of adult tonsillectomy were recovered by net cost savings after tonsillectomy at 12.7 years after tonsillectomy (break-even point). The overall economic costs (medical costs and work-related costs) were recovered at 2.3 years after tonsillectomy.

Sensitivity Analysis. The economic model's sensitivity to various cost factors and assumptions was assessed by varying these parameters and constructing new break-even models. The overall economic break-even point was found to be most sensitive to the discount rate. Increasing the discount rate to 7.5% moved the break-even point to 2.5 years from surgery (see Figure, B). The overall economic model was not sensitive to changes in the CPI-M or the reimbursement rate for physician visits. Increasing the CPI-M to 8% moved the medical cost break-even point up to 11.4 years, but had little effect on the overall economic break-even point. The break-even point was found to be most sensitive to the number of workdays missed because of episodes of tonsillitis.

DISCUSSION

Tonsillectomy is one of the most commonly performed procedures in medicine.⁸ Traditionally, indications for tonsillectomy have included chronic recurrent tonsillitis and/or pharyngitis and upper airway obstruction.⁹ Less common indications for tonsillectomy include chronic halitosis, cryptic debris, and the persistent streptococcal carrier state. Despite



Break-even analysis for adult tonsillectomy with discount rate at A) 5% and B) 7.5%.

the extirpative nature of tonsillectomy, patients are not necessarily cured of their recurrent tonsillitis and/or pharyngitis episodes. Therefore, in reality, tonsillectomy provides a quality-of-life improvement in terms of the frequency and severity of pharyngitis episodes, especially when performed for an indication of chronic tonsillitis.

Therefore, in order to fully assess the clinical benefit of tonsillectomy, quality-of-life improvement needs to be determined. In addition, to more thoroughly determine the true benefit of tonsillectomy, economic factors should be considered. One such method of economic evaluation of a surgical procedure is "break-even" analysis. This method attempts to quantify the "up-front" cost of an intervention and then determine when the cost of that intervention is recovered by the subsequent decrease in medical and economic expenditures due to improvement of the disease state from the intervention. Break-even time analysis is not a new concept to medicine, and has been used to estimate the fiscal impact and economic improvement from various procedures, including surgical treatment for gastroesophageal reflux disease, endoscopic sinus surgery, implantable cardiac defibrillators, endoscopic colorectal cancer screening, and renal transplantation.^{5,10-12} For example, Gliklich and Metson⁴ found that endoscopic sinus surgery resulted in a medical break-even point of 15 years and an overall economic break-even point (including savings from work-related disability) of approximately 7 years. Often, more serious medical conditions result in shorter break-even periods. Renal transplantation has been found to reach a break-even point as early as 1.7 years after transplantation when the costs of continued dialysis are avoided.¹² Implantable cardiac defibrillators used as a life-saving measure reach a break-even point in approximately 1 to 3 years af-

ter implantation.⁵

This study was specifically undertaken to determine the economic impact of chronic tonsillitis in the adult patient population and the medical and economic improvements resulting from adult tonsillectomy. Such information is potentially useful in counseling patients and justifying tonsillectomy in the face of an ever-shrinking health-care dollar. Gathering data regarding tonsillectomy outcomes in the adult population is not without difficulty. We were somewhat disappointed in the relatively poor response rate. This was not surprising, given that the majority of our patients undergoing adult tonsillectomy are in their third decade, often in secondary school or college, and are therefore highly likely to relocate within a few years after tonsillectomy. We attempted to locate all patients after returned, undelivered first mailings by searching billing and hospital record databases and sending a second mailing. Therefore, the response rate reflects results after 2 mailings in an attempt to gather follow-up data.

Although we were not surprised by the relatively high number of weeks on antibiotics in the 12 months prior to tonsillectomy, we were surprised that patients missed approximately 9 days of work because of tonsillitis exacerbations. This work-related disability results in a significant economic cost: well over \$1,000/y. This economic cost easily overshadows the average expenditure on antibiotics and physician visits due to chronic tonsillitis prior to tonsillectomy. However, the combined antibiotic and physician visit costs together account for a significant health-care expenditure, approximating \$250/y. It is possible that the antibiotic costs would be greater if longer courses of antibiotics were attempted or more expensive antibiotics, such as second-generation cephalosporins, macrolides, or fluoroquinolones, were used. We elected

to use the most inexpensive first-line antibiotic agents in our model so as to produce a conservative estimate of the break-even point.

It is clear that adult tonsillectomy results in a significant decrease in number of weeks on antibiotics, number of physician visits for chronic tonsillitis, and number of workdays missed because of chronic tonsillitis. Each of these reductions results in a corresponding cost savings either to the medical management of chronic tonsillitis or to the economy as a whole. In addition, such reductions in health-care utilization have other, intangible benefits. For example, decreases in the number of weeks on antibiotics may presumably result in decreased antibiotic resistance rates in the future, which would add additional cost savings. A decrease in the number of physician visits for chronic tonsillitis allows other patients to use those clinic slots, resulting in potentially improved efficiency of health-care utilization. Given that many patients with chronic tonsillitis may be treated by telephone rather than by office visit, decreased use of antibiotics also probably reflects an intangible medical cost savings with respect to physician telephone time.

We believe that the relatively short break-even point of approximately 2.3 years for the recovery of tonsillectomy costs by savings in medication expenditures, physician expenditures, and the cost of work-related absence reflects very favorably on the cost-effectiveness of adult tonsillectomy for chronic tonsillitis. Our economic valuation for the cost of tonsillectomy is very similar to that determined by a study of a large insurance database that found that the average medical cost for tonsillectomy approximated \$2,900 in the early 1990s.¹³ Despite these costs, given the significant positive impact that tonsillectomy has in reducing work-related absences, the impact on the patient's work environment and job status should be considered in evaluating patients for adult tonsillectomy. In addition to the frequency of tonsillitis episodes, the number of days of work missed, the number of physician visits, and the number of weeks of antibiotics required should be considered in the decision for tonsillectomy. In a study by Roos et al,¹⁴ the average cost of a single tonsillitis episode, including medical cost of evaluation and treatment and lost work productivity, was \$380. They, too, found that the work-related costs of the tonsillitis episode were more substantial than the cost of medical treatment. Given that the break-even time analysis was most sensitive to the number of workdays missed because of tonsillitis, work absences should be included when evaluating the economic benefit derived from adult tonsillectomy.

Several elements of the data analysis and data selection for this study merit comment. First, this study methodology utilizes medical care data for the two 12-month periods before and after tonsillectomy, and extrapolates that out to several years after tonsillectomy in order to calculate the break-even time analysis. It is possible that medical care utilization rates could fluctuate as time extends from the initial diagnosis. However, given that chronic tonsillitis is a chronic, persistent condition, one would expect such fluctuation to be somewhat limited. Use of a 12-month time period for cost-of-disease assessment has a precedent in the literature.⁴ Also, there may be hidden medical costs that were not included in the cost assessment, such as throat cultures or oral pain medication for acute exacerbations of tonsillitis. Such unaccounted-for costs in the medical management of chronic tonsillitis would tend to make the break-even point for the medical costs segment arrive sooner. Similarly, we chose not to include the potential costs of the management of post-tonsillectomy bleeding, because in many instances, this complication does not require hospitalization or a follow-up procedure. In addition, such management, especially in the outpatient setting, would likely be included in the global fee for tonsillectomy. Finally, we chose to include only first-line antibiotics in the cost analysis for tonsillitis. These antibiotics were selected because they are the recommended first-line agents for acute tonsillitis and pharyngitis. We did find that the economic model was moderately sensitive to the price of antibiotics when the price of daily therapy exceeded \$4.00, as could be expected with second-generation cephalosporins or fluoroquinolones. Although it is possible to construct more detailed and intricate economic models for chronic tonsillitis and tonsillectomy, we believe that the present model includes the most salient medical practice parameters in the decision-making process for tonsillectomy, and therefore provides accurate insight into the economic impact of chronic tonsillitis and tonsillectomy.

CONCLUSIONS

Chronic tonsillitis in the adult population carries with it significant medical and overall economic expenditures. Tonsillectomy can be reasonably expected to result in substantial decreases in health-care utilization and work-related absences. These health-care and economic improvements after tonsillectomy create cost savings that result in acceptable break-even points when tonsillectomy is considered as an "investment" toward anticipated improvements in health status. Such improvements should be considered in the clinical decision-making process for tonsillectomy.

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EUROPEAN ACADEMY OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY

The Congress of the ENT Section of the European Academy of Allergology and Clinical Immunology will be held in Ghent, Belgium, November 15-18, 2003, and will be accompanied on November 17-19, 2003, by the Fifth International Symposium on Experimental Rhinology and Immunology of the Nose. For further information, contact Congress Secretariat, Semico nv, Korte Meer 16, B-9000 Ghent, Belgium; telephone +32 9 233 86 60; fax +32 9 233 85 97; e-mail eaaci@semico.org or see the web site <http://www.semico.org/serin>.

SUDDEN SENSORINEURAL HEARING LOSS: SUPPORTING THE IMMUNOLOGIC THEORY

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Sudden deafness constitutes a diagnostic challenge. Classically, 2 causes, viral and vascular, are considered in the origin of idiopathic sudden hearing loss. More recently added to the list of possibilities are rupture of the membranous labyrinth and immune-mediated sensorineural hearing loss. The latter can be either primary and localized to the inner ear or, in perhaps fewer than one third of cases, secondary to generalized systemic autoimmune disease. The purpose of the present review is to define immune-mediated sudden sensorineural hearing loss as a distinctive entity, on the basis of clinical, immunologic, and pathological findings, and suggest a profile of the typical patient.

KEY WORDS — immunopathology, inner ear disorders, sudden deafness.

Sudden deafness may be defined as a sensorineural hearing loss (SNHL) of 30 dB or more over at least 3 contiguous audiometric frequencies that develops over a period of a few hours to 3 days.¹ Its cause can be found in only 10% to 15% of patients.² Sudden hearing loss should be considered a medical emergency, because of hearing's importance to communication. The estimated yearly incidence of sudden hearing loss is 5 to 20 cases per 100,000 persons.³ By definition, idiopathic sudden hearing loss is a diagnosis of exclusion only after a thorough search for known causes (Table 1).

The prognosis is highly variable, depending on the severity and pattern of initial loss, the interval between onset and presentation, and the presence of vertigo. Haberkamp and Tanyeri⁴ wrote that no consensus exists on treatment.

Immune-mediated inner ear disease has been added to the classic triad of viral infection, vascular disorders, and rupture of the membranous labyrinth as causes of sudden deafness. It was first reported as a clinical entity in 1979 by McCabe,⁵ on the basis of a clinical profile and response to steroids and cyclophosphamide. Randomized, double-blind, placebo-controlled, clinical trials have reported a 78% recovery rate with steroids for patients with moderate hearing loss (35 to 90 dB), versus 38% for placebo,¹ suggesting the role of the immune system in sudden hearing loss. A relationship among vascular disorders, viral infection, and immune-mediated causes of sudden hearing loss has been demonstrated in animal models of experimental labyrinthitis, in which a viral infection led to abnormalities of cochlear flow and a

strong immunologic response within the inner ear with the production of a fibro-osseous matrix that was responsible for irreversible cochlear injury.⁶ Such alterations are similar to those described in diverse systemic autoimmune diseases, in many of which a viral infection can induce the autoimmune response.⁷ However, several differences can be established between sudden hearing loss (unilateral presentation in less than 72 hours) and autoimmune inner ear disease (AIED; bilateral progressive presentation in days to 3 months).⁸ A positive response to immunosuppressive therapy supports an immune-mediated origin.

The existence of a local immune response within the inner ear, which may originate in and be enhanced in the endolymphatic sac (ELS), with recruitment of immunocompetent cells from the bloodstream, has been experimentally demonstrated.⁹⁻²⁴ Sudden hearing loss can be the first manifestation of systemic autoimmune diseases such as Cogan's syndrome,²⁵ temporal arteritis,²⁶ Wegener's granulomatosis,²⁷ polyarteritis nodosa (PAN),²⁸ systemic lupus erythematosus (SLE),²⁹ and others (Table 2). However, evidence of the relationship requires fulfillment of the Witebsky postulates, that is, a modification of Koch postulates designed for immunologic phenomena: association of autoimmunity with the clinical state; identification of the autoimmune response; transferability of the response, using lymphoid cells or serum transfer, to an animal model; and re-creation of the disease and clinical state in that model by such a transfer. Unfortunately, these criteria cannot be applied to sudden hearing loss and rapidly progressive

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TABLE 1. KNOWN CAUSES OF SUDDEN SENSORINEURAL HEARING LOSS

Cochlear causes

Infectious causes: viral agents (mumps, rubeola, measles, cytomegalovirus, herpesvirus: simplex, varicella-zoster, Epstein-Barr), syphilis, *Mycoplasma pneumoniae*, Lyme disease, toxoplasmosis

Traumatic causes: temporal bone fracture, inner ear decompression sickness, inner ear concussion, otologic surgery, perilymph fistula, lumbar puncture

Circulatory causes: Buerger's disease, slow blood flow of vertebrobasilar system, sickle cell disease, cardiopulmonary bypass, mitochondriopathy, red blood cell deformability, leukemia, myeloma

Systemic autoimmune diseases: polyarteritis nodosa, Cogan's syndrome, systemic lupus erythematosus, rheumatoid arthritis, Wegener's granulomatosis, relapsing polychondritis, temporal arteritis, dermatomyositis and polymyositis, scleroderma

Endolymphatic hydrops, including Meniere's disease

Metabolic causes: renal failure, diabetes mellitus, hypothyroidism

Ototoxicity: aminoglycoside antibiotics, loop diuretics, salicylates, nonsteroidal anti-inflammatory drugs, vancomycin, erythromycin, azithromycin, oral contraceptives, interferon, quinine and derivatives, cisplatin

Retrocochlear and central nervous system causes

Meningitis

Sarcoidosis

Multiple sclerosis

Amyotrophic lateral sclerosis

Friedreich's ataxia

Guillain-Barré syndrome

Vogt-Koyanagi-Harada syndrome

Xeroderma pigmentosum

Tumors: acoustic neuroma, carcinomatous neuropathy, metastases in cerebellopontine angle

Central deafness: Wallenberg's syndrome, cortical encephalitis, Alzheimer's disease

SNHL.³⁰ Numerous attempts have been made to develop assays to detect serum markers that might help to confirm the diagnosis and identify patients affected by this condition (Table 3).

PATHOPHYSIOLOGY

A significantly increased incidence of major histocompatibility complex (MHC) class II genes, elevated levels of circulating immune complexes, specific and nonspecific inner ear autoantibodies, and abnormal T lymphocyte subpopulations in patients affected by sudden hearing loss all suggest an immune-mediated causation and support the role of sudden deafness as a clinical presentation of AIED.

Major Histocompatibility Complex. The association of human leukocyte antigen (HLA) with particular diseases is one of the most intriguing aspects of the MHC, which is located on the short arm of chromosome 6 in humans. Although the MHC was originally identified by its role in transplant rejection, it is now recognized that proteins encoded by this region are involved in immunologic recognition and interaction between lymphocytes, as well as between lymphoid cells and antigen-presenting cells (APCs).

The MHC is divided into 3 regions: the class I region encodes for molecules found on nucleated cells and includes the A, B, and C gene loci; the DR, DP, and DQ genes encode the alpha and beta chains of the class II proteins, expressed on macrophages, Langerhans cells of the skin, B cells, and activated T

cells; the class III genes encode for some proteins of the complement pathway and proteins involved in inflammation and immune regulation such as heat shock protein 70 (HSP-70) and tumor necrosis factor alpha (TNF- α).³¹

The MHC class I molecules, which present peptides to CD8 T cells, bind peptides from proteins degraded in the cytosol. The MHC class II molecules, which present peptides to CD4 cells, bind peptides from proteins degraded in acidified intracellular vesicles. These two MHC molecules deliver peptides from cellular compartments to the cell surface, where they are recognized by T cells that mediate effector functions. Polymorphism is the most important characteristic of HLA molecules, and the peptide motifs presented by HLA are dependent upon individual alleles. Some autoimmune diseases (SLE, myasthenia gravis, and Graves' disease) may be influenced by inherited alleles, which may play a key role in auto-antigen presentation.

Polymerase chain reaction has established a correlation between HLA genotypes and inner ear diseases such as idiopathic SNHL and Meniere's disease. The presence of HLA-DRB1 14 in patients with sudden SNHL has been reported, suggesting that HLA-DRB1 04 may be a useful marker for predicting a poor prognosis.³²

Immunopathology. Although the inner ear was initially considered an immunologically privileged organ, it is now recognized that it is capable of strong

TABLE 2. AUTOIMMUNE DISORDERS ASSOCIATED WITH SUDDEN SENSORINEURAL HEARING LOSS

Connective tissue diseases
Systemic lupus erythematosus
Rheumatoid arthritis
Polyarteritis nodosa
Takayasu's disease
Wegener's granulomatosis
Polymyositis and dermatomyositis
Progressive systemic sclerosis
Mixed connective tissue disease
Sjögren's syndrome
Cogan's syndrome
Behçet's disease
Relapsing polychondritis
Endocrine disorders
Hashimoto's disease and Graves' disease
Hematologic diseases
Acquired immune hemolytic disorders
Autoimmune thrombocytopenic purpura
Other diseases
Glomerulonephritis
Myasthenia gravis
Demyelinating diseases
Uveitis
Ulcerative colitis
Sarcoidosis
Immunodeficiency diseases
Allergy

Modified from Veldman JE. Immune-mediated inner ear disorders: new syndromes and their etiopathogenesis. In: Veldman JE, McCabe BF, eds. *Oto-immunology*. Amsterdam, the Netherlands: Kugler, 1987:125-41.

immune responses. These responses may be mediated by immunocompetent cells recruited from the ELS or the systemic circulation. Inflammatory injury may be directed against inner ear antigens by nonspecific bystander mechanisms or by the ischemic effects of vasculitis.

The antigens that reach the cochlea rapidly gain access to the immune system. When an antigen is situated in the inner ear of animals that have previously been sensitized by a systemic route, an immune response consisting of cellular infiltration, inflammation, and a cochlear lesion is produced, and there is an increase in the titer of perilymphatic antibodies and local antibody production. The accumulation of leukocytes and the local production of immunoglobulins in the inner ear depends on the entrance of leukocytes from the bloodstream, facilitated by the release of mediators of the immune response such as interleukin-2, which may originate in the ELS. This response activates the endothelial cells of the spiral modiolar vein, which assume features of high endothelial venules, potentiating the expression of intercellular adhesion molecule 1, which recruits leuko-

TABLE 3. IMMUNOLOGIC AND MICROBIOLOGICAL WORKUP FOR PATIENTS WITH IMMUNE-MEDIATED INNER EAR DISEASE

Immunologic battery
Erythrocyte sedimentation rate, serum immunoglobulins IgG, IgA, IgM, complement factors C3, C4
Autoantibodies: antinuclear antibodies, extractable nuclear antigen
Rheumatoid factor
Immunophenotype of peripheral blood lymphocytes
Western blot for heat shock protein-70
Microbiological study*
Syphilis: VDRL test, fluorescent treponemal antibody absorption test
<i>Mycoplasma pneumoniae</i>
Epstein-Barr virus
Herpes simplex virus
Varicella-zoster virus
Cytomegalovirus
Influenza: A, B
Parainfluenza: 1, 2, 3
Adenovirus
Mumps

*Microbiological study is performed only in patients previously affected by upper respiratory tract infection (<3 weeks).

cytes from the bloodstream. There is a concomitant formation of a dense extracellular matrix with considerable fibrosis that, since it cannot be reabsorbed, triggers ossification. The immune response can provoke the degeneration of the organ of Corti, stria vascularis, and spiral ganglion.³³

Hearing loss can be the first manifestation of systemic autoimmune diseases such as Cogan's syndrome, PAN, and SLE. Although the vascular damage frequently observed in SLE and other vasculitides is probably mediated by immune complexes, autoantibodies against endothelial cells may damage endothelial cells via a complement-mediated or antibody-dependent cellular cytotoxic mechanism, or by upregulation of adhesion molecules and proinflammatory cytokines.³⁴ The breakdown of tight junctions between stria vascularis endothelial cells, and consequently of the blood-labyrinth barrier, may be one of the etiologic mechanisms of SNHL in autoimmune disease.

Viral Infection. Oldstone⁷ has identified 3 lines of evidence suggesting a possible relationship between viruses and autoimmune disease: first, autoimmune responses are induced or enhanced by infection with a wide array of human DNA and RNA viruses; second, in animal models acute and persistent viral infections induce, accelerate, or enhance autoimmune responses and initiate autoimmune diseases; and third, viruses can potentiate autoimmune responses as a re-

sult of similar antigenic determinants between viruses and host components. After viral infection, the shared epitopes produce cross-reacting immune responses that can initiate autoimmune disease. An effector immune response, humoral (B cell) and/or cytotoxic (T cell), is directed against a virus and may cross-react with self protein or autoantigen, evoking an autoimmune response. As a result of molecular mimicry between viruses and inner ear tissues, humoral and/or cellular immune responses could cause auditory and/or vestibular disorders.

Alterations in T cell subpopulations have been reported in patients with sudden deafness of possible infectious cause (respiratory viruses and *Mycoplasma pneumoniae*), showing an increase in T memory cells (CD4CD45RO) and a decrease in naive T cells (CD4CD45RA) suggesting that the infectious agent facilitates an antigenic stimulus strong enough to transform the naive cells into memory cells or that there is a change in the cytotoxic effect of the naive cells, which would also be conducive to a permanent state of infection.³⁵ The activation of the virus after latency and/or the onset of a second viral infection caused by the same agent after a state of chronic sub-clinical infections could recruit a large number of memory T cells, resulting in an immune response so intense that it would produce a marked cytopathic effect in the inner ear, leading to a structural change that could provoke irreversible lesions in some cases.

Temporal Bone Histopathology. Histopathologic studies of temporal bones from patients with systemic autoimmune diseases affected by sudden deafness showed cochlear fibrosis and ossification in the inner ear, suggesting the vascular insult theory.^{36,37} Atrophy of the organ of Corti and stria vascularis is also observed in cochleas from patients with SLE and PAN. Two of 4 cases of PAN showed occluded labyrinthine arteries with all layers of the arterial wall thickened and complete degeneration of the internal elastic membrane; however, the vessel walls contained no acute or chronic inflammatory cells.³⁸ Thickened blood vessels adjacent to the ELS were reported in Wegener's granulomatosis.³⁹ Severe fibrosis of the entire inner ear, marked inflammatory cell infiltrate, and mild vasculitis of the middle ear were reported in a young patient with SLE.³⁶

Endolymphatic hydrops, proliferation of fibrous tissue and bone filling the perilymphatic spaces, severe atrophy of the organ of Corti and stria vascularis, and retrograde neuronal degeneration characterize immune-mediated inner ear disease.⁴⁰ These changes are consistent with an inflammatory and possibly ischemic attack on the membranous labyrinth.

Autoantibodies. The existence of autoantibodies

against inner ear antigens implies an autoimmune pathogenesis of sudden hearing loss. However, microthrombosis of the inner ear may result from pathogenic anticardiolipin antibody activity, thereby playing a role. Anticoagulant therapy is included in the majority of treatment protocols for sudden deafness.⁴¹ The presence of antiendothelial antibodies in patients with sudden hearing loss supports immune-mediated vascular damage in which antiendothelial antibodies may have a pathogenetic role and may represent a serologic marker of vasculitis.³⁴ Studies on antinuclear autoantibodies (ANAs), rheumatoid factor, antimitochondrial autoantibodies, antibodies against smooth muscle, antisarcolemmal antibodies, antilaminin antibodies, and antibodies against type II collagen⁴²⁻⁴⁴ yielded controversial results. On the basis of these serologic findings, we only include ANAs in our immunologic workup; high ANA titers have been frequent in this group of patients.⁴⁴ The presence of ANAs does not rule out systemic autoimmune diseases, in which cochleovestibular dysfunction could be the first manifestation of disorders such as SLE²⁹ and Sjögren's syndrome.⁴⁵

Complement activation study might identify patients with immune-mediated sudden deafness. The complement system consists of approximately 30 proteins that act synergistically to induce an inflammatory reaction; it also represents an important barrier against infections. Because of its inflammatory effect, it also can contribute to tissue destruction in a variety of autoimmune and inflammatory diseases not necessarily caused by infective agents. Complement can be activated through either classic or alternative pathways; either way, factor C3, the central component in the first part of the cascade, is activated. Elevated levels of the activation product of C3, C3bc, have been detected in patients with sudden deafness,⁴⁶ indicating an immune cause and thus identifying patients who would benefit from anti-inflammatory drugs. We have not observed these findings, and our serologic determinations were performed in the first 24 hours after the hearing loss. Perhaps the activation of the complement system expresses a systemic inflammatory reaction in which inner ear dysfunction is the only clinical manifestation. This theory could support the lack of symptoms of a systemic reaction. These results do not support the presence of active infection as suggested by Pitkaranta and Julkunen.⁴⁷ They studied the presence of Mxa protein in patients' leukocytes, but were unable to demonstrate an association between systemic viral infection and onset of sudden hearing loss. This local reaction can stimulate host immune responses, leading to pathological changes via immunopathologic reactions or by triggering an autoimmune reaction.

Measurements of total hemolytic component (CH50), component factors C3, C4, and C1q, immunoglobulins IgG, IgA, and IgM, C-reactive protein, and erythrocyte sedimentation rate would support the diagnosis of immune-mediated sudden hearing loss, particularly in cases associated with systemic autoimmune disease.^{48,49}

Western Blot Immunoassay. Attempts at finding a specific diagnostic marker for AIED were initially promising. An anti-68 kd autoantibody in the sera of patients with rapidly progressive SNHL was demonstrated by Western blot analysis.⁵⁰ Evidence that links the 68 kd antigen with highly inducible heat shock protein 70 (HSP-70) was reported.⁵¹ Heat shock proteins, which are believed to protect cells by dissolving and refolding misfolded or denatured protein, are induced by various forms of stress, including heat, ischemia, toxic agents, free radicals, and acoustic overstimulation. This protein is present in the spiral limbus, spiral prominence, stria vascularis, and organ of Corti (supporting, pillar, outer hair, and interdental cells); this antigen is also recognized in brain, intestine, liver, kidney, and muscle.⁵²

Antibodies to HSP-70 have been identified in systemic autoimmune disorders such as ulcerative colitis.⁵³ This antibody test could detect a local autoimmune process within the inner ear in the absence of systemic involvement. However, hearing loss could be the first manifestation of a systemic autoimmune disease; therefore, interpreting the results is difficult. We do not know whether reactivity to HSP-70 is only a marker for AIED or whether it plays a more determinant role in the pathogenesis.

The low percentage of positive HSP-70 findings in patients with sudden deafness⁵⁴ suggests that the inner ear response is basically cellular, as has been observed in several animal models, with recruitment of inflammatory cells toward the cochlea. This fact, together with the sudden form of presentation, would prevent the genesis of lymphocytes secreting anti-HSP-70 antibodies. However, the presence of systemic autoimmune diseases represents a limitation to the diagnosis of this entity by Western blot immunoassay.⁵ Thus, the diagnosis of AIED is still based on the clinical form of presentation and on the response to therapy. Flow cytometry has been incorporated into the immunologic workup with the objective of demonstrating alterations of the T-cell subpopulations. The typical clinical course, immunologic changes, and response to steroid therapy are indicative of this entity.

Phenotypic Analysis of Peripheral Blood Lymphocytes. The association of immune-mediated mechanisms with rapidly progressive SNHL has been men-

tioned in numerous studies,^{50,55-58} but few have addressed their causative role. Few studies have identified the lymphocyte subpopulations in patients' peripheral blood samples.

Abnormalities in CD4+ and CD8+ subpopulations and the CD4+/CD8+ ratio were observed by use of 3-color flow cytometry,⁵⁹ suggesting that sudden deafness could present as an immunodeficiency disorder. Our investigations showed decreased numbers of both CD4+ helper cells and CD8+ cytotoxic cells, as well as reduced numbers of naive T cells (CD4+/-CD45RA+ and CD8+CD45RA+). Patients with a positive response to steroid therapy showed a tendency toward normalization of these subpopulations after treatment; however, patients with a poorer response had increased memory T cell (CD4+/-CD45RO+) and low naive T cell (CD8+CD45RA+) levels. These differences may indicate the persistence of the antigen that triggers the immunologic mechanism, given the possible transformation of CD4+ naive cells into memory cells that would continue despite treatment. Moreover, a population of deficient naive cytotoxic cells could suggest an a priori poor response to steroid therapy due to a deficiency of cytotoxic mechanisms in response to the triggering antigen.

T lymphocytes have been divided into naive cells and memory cells that respond to a recall antigen. As lymphocytes develop into memory cells after antigenic stimulation, they cease to express CD45RA and begin to express CD45RO on their surface. Naive and memory phenotypic T cells have different properties. Naive T cells are especially potent in mediating responses to alloantigens (eg, causing skin graft rejection and induction of graft-versus-host disease). Naive T cells also aid in primary antibody responses. Memory T cells provide strong T-helper function for B cells and secondary T-cell proliferative responses to antigen.

The basis for T-cell abnormalities in patients with sudden deafness is unclear, but may relate to the autoimmune or immune-mediated nature of the disease. We do not know whether the immune system is causing the ear damage or reacting to it. These abnormalities are similar to those observed in other autoimmune diseases such as SLE⁶⁰ and Behçet's disease.⁶¹ Although the causes of autoimmune diseases may differ, there appear to be similarities that are related to alterations of immune regulation. A decrease in CD4-CD45RA cells could be due to autoantibodies to CD45RA molecules, due to the in vivo conversion of naive cells to memory cells as a result of an ongoing immunologic response, or caused by the viral infection of these cells, although according to our

study, this hypothesis would be improbable, since few patients presented with upper respiratory tract infection of probable viral origin.³⁵ An alternative explanation could be a preferential homing of naive cells to focal inflammatory sites (inner ear). The role of T lymphocytes has been reported in animal models of autoimmune experimental labyrinthitis.^{20,21} These studies support the role of immunocompetent cells in the pathogenesis of sudden deafness, but the antigen or precise initiating agent has yet to be identified.

MAGNETIC RESONANCE IMAGING

Although the first objective of magnetic resonance imaging (MRI) in patients with sudden deafness is to rule out tumors (cerebellopontine angle, internal auditory canal, ELS, vestibule, and cerebral tissue), this technique has occasionally facilitated the diagnosis of immune-mediated inner ear disease. Hyperintensity within the membranous labyrinth on pre-contrast T1-weighted images has been reported in a patient with Cogan syndrome²⁵ and probably represents hemorrhage and leakage through an abnormal labyrinthine membrane due to active disease (inflammation of stria vascularis blood vessels).²⁵

A correlation between hyperintensity extending to the vestibule and posterior labyrinth and a poor recovery of hearing loss was established.

Another study reported that the cochlea and/or the vestibule showed higher signal intensity on proton density and T2-weighted images on the diseased side (MRI-positive cases), suggesting changes in the chemical composition of the perilymph and/or endolymph, since proton density and T2-weighted images reflect water content.⁶² The MRI-positive sudden deafness cases showed a poorer prognosis than did MRI-negative cases.

Fitzgerald and Mark⁶³ observed that patients with immune-mediated sudden hearing loss had a high frequency of abnormal MRI findings (66%). These authors support early contrast-enhanced MRI to provide an anatomic basis for therapy.

Unfortunately, when the diagnosis of immune-mediated sudden deafness is delayed, extensive soft tissue obliteration occurs inside the inner ear, as seen on T2-weighted images. This has been found in inflammatory and/or ischemic injury to the labyrinth, as in Cogan's syndrome. Long-term disease shows bony or calcified obliteration on high-resolution computed tomography that is similar to that in labyrinthitis ossificans.⁶⁴

DIAGNOSTIC PROFILE

On the basis of immunologic studies, we propose

a profile of immune-mediated sudden SNHL.^{65,66} Major criteria would include 1) bilateral involvement, 2) systemic autoimmune disease, 3) high levels of ANAs, 4) a reduced number of naive T cells (CD4+RA), and 5) a more than 80% rate of recovery of hearing. Minor criteria would include 1) unilateral involvement, 2) a young or middle-aged patient, often female, 3) serum reactivity against HSP-70, and 4) a positive response to steroid treatment (recovery rate <80%). Three positive major criteria or 2 major criteria plus 2 minor criteria would support an immune-mediated disorder, taking into account that profound hearing losses (>90 dB) present a low percentage of recoveries, regardless of the cause.

The analysis of new cochlear proteins and the administration of new therapeutic protocols will lead future research.

THERAPY

Corticosteroids are widely used in the treatment of sudden SNHL and have been the standard care in treating AIED. They exert their effect through potent suppression of the immune-mediated inflammatory response (by blocking autoantibody production, interleukins IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and IL-8, TNF- α , interferon- γ , granulocyte-macrophage colony-stimulating factors, and lymphoproliferation), by decreasing capillary permeability, by stabilizing lysosomal membranes, by blocking migratory inhibitory factors, and by inhibiting arachidonic acid metabolism. A role of corticoids in the regulation of ionic and fluid dynamics may exist, since the sodium, potassium adenosinetriphosphatase (Na,K-ATPase) activity and Na,K-ATPase sites of the lateral wall of the inner ear are modulated in accord with the presence or absence of circulating glucocorticoids; corticosteroid administration correlates with increased levels of Na,K-ATPase in the inner ear.⁶⁷⁻⁶⁹

We initiated therapy with 6-methylprednisolone at 1 mg/kg body weight per day; this dose was tapered during the next 21 days. In patients with profound hearing loss (>70 dB), a daily 500- to 1,000-mg bolus of 6-methylprednisolone was administered for 3 days. Patients who were nonresponsive or had recurrent disease started therapy with methotrexate (MTX); oral MTX was begun at a dose of 7.5 mg/wk and in most cases was increased to 15 mg/wk given in 2 divided doses to achieve maximum response, over 4 to 8 weeks.^{44,70} At monthly intervals, complete blood count and liver enzyme testing were used to assess for toxicity. To minimize toxicity, we prescribed folic acid at a dose of 1 mg/d.^{71,72} The criteria for a response to immunomodulatory therapy included a recovery of hearing of more than 60%. Serial audiograms were obtained for 1 year.

Corticosteroid administration directly into the inner ear through the membrane of the round window⁷³ makes it possible to achieve elevated concentrations and avoid the collateral systemic effects of these drugs. The mechanism of action of steroids in the inner ear remains open to speculation; they increase the microvascular blood flow in the cochlea, reduce the inflammation and the onset of endolymphatic hydrops, and lack ototoxic effects in laboratory animals.

Although the drug is delivered directly to the inner ear, the lack of consensus with respect to the dose to be administered and the duration of treatment, and the presence of a communication through the tympanic membrane that facilitates the spread of microorganisms from the outer ear to the middle ear and even the inner ear, increasing the risk of labyrinthitis, has

led to the study of designs for drug delivery devices that allow controlled chronic administration of different substances.

Drug Delivery Devices. The development of systems for drug delivery and the administration of antioxidants, growth factors and protease inhibitors, and HSP-70 opens new options in the treatment of hearing loss produced by noise and by ototoxic drugs. Certain antioxidants (adenosine agonists, glutathione derivatives) have been administered directly into the round window.

The direct administration of immunosuppressive drugs into the inner ear by means of drug delivery devices (osmotic pumps) may facilitate the treatment of immune-mediated diseases of the inner ear, especially in patients who do not initially respond to conventional therapy.

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BLOOD FLOW MEASUREMENTS IN THE EARS OF PATIENTS RECEIVING COCHLEAR IMPLANTS

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We measured cochlear blood flow in 12 patients who received cochlear implants, using a laser-Doppler probe with an outer diameter of 0.8 mm. The subjects had congenital deafness, idiopathic progressive sensorineural hearing loss, Waardenburg's syndrome, narrow internal auditory canal, or sudden deafness. Putting the probe tip to the site of drilling for cochlear implantation, we measured blood flow before, during, and after the cochlear bony wall was opened. The laser-Doppler output was confirmed even after the tip of the probe was inserted into the perilymphatic space in all cases. Our results revealed that blood flow was maintained in all cochleas, although there was a probability of reduction in blood flow volume. We conclude that laser-Doppler flowmetry is both relatively safe and useful for measuring blood flow in the ears during cochlear implantation procedures.

KEY WORDS — cochlear blood flow, cochlear implant, deafness, laser-Doppler flowmetry, narrow internal auditory canal, Waardenburg's syndrome.

INTRODUCTION

Impairment of cochlear blood flow (CBF) may be one of the factors implicated in the pathophysiology of sensorineural hearing loss.^{1,2} To evaluate CBF, various methods have been used, including intravital microscopy,^{3,4} impedance plethysmography,⁵ laser-Doppler flowmetry,^{1,6} and microspheres.^{7,8} However, at present, only laser-Doppler flowmetry is clinically available for measuring human CBF.^{1,9-14} For this approach, the tip of a probe is normally placed on the bony promontory over the cochlea. In this situation, the degree of contribution of the bone blood flow to the laser-Doppler output must be evaluated. Laser-Doppler flowmetry gathers more signals from the blood flow in the area closest to the tip of the probe, and the bone's blood supply, which probably comes from the external carotid artery system, may behave differently from the CBF, which comes from the vertebral artery system.¹⁵ At present, the degree of contribution of the bone blood flow to the laser-Doppler output in humans is not clear.

When assessed by laser-Doppler flowmetry, the degree of contribution of the bone blood flow is associated with the thickness of the bone, the wavelength of the laser, the permeability of the bone, the size of the probe, and the position of the probe tip.^{10,16,17} On the basis of results of simultaneous measurements using laser-Doppler flowmetry and microspheres in rats, it has been estimated that be-

tween 30% and 40% of the laser-Doppler output depends on bone blood flow.¹⁸ Because the bone surrounding the cochlea is thicker in humans than in experimental animals, the actual contribution of the bone blood flow to the laser-Doppler output is likely to be greater in clinical situations.

Patients receiving cochlear implants offer clinicians a unique opportunity to evaluate the contribution of the bone blood flow systematically, as the bone surrounding the cochlea is opened. We believe that the true contribution of the CBF can thus be measured most accurately by laser-Doppler flowmetry in such a situation. Here we report on the measurement of CBF in the ears of patients receiving cochlear implants.

METHODS

The subjects for this investigation were 12 patients who received cochlear implants. There were 7 male patients and 5 female patients, and their ages ranged from 2 to 55 years. All parents of the children and all adult patients received an explanation of the method and purpose of the study, and provided informed consent. The causes of deafness were unknown, except in 1 patient, who had Waardenburg's syndrome. One child had bilateral narrow internal auditory canals (Fig 1). Three adults had been found to have idiopathic progressive sensorineural hearing loss. One adult patient had experienced idiopathic sudden sen-

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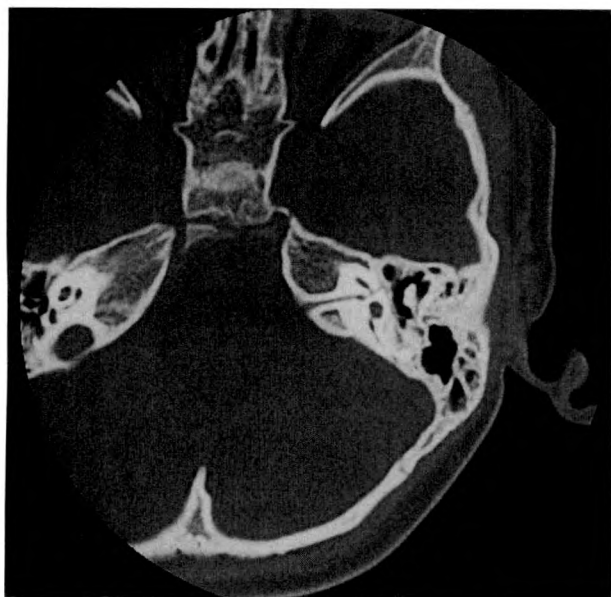


Fig 1. Computed tomogram of case of bilateral narrow internal auditory canals.

sorineural hearing loss 2 years previously.

The CBF was measured with a laser-Doppler flowmeter (model ALF 21, Advance, Tokyo, Japan) under general anesthesia with sevoflurane and nitrous oxide. The systolic blood pressure was between 85 mm Hg and 123 mm Hg and the diastolic blood pressure was between 35 mm Hg and 70 mm Hg at measurement. The outer diameter of the probe was 0.8 mm, and the fiber separation between the exciting and receiving optic fibers was 0.3 mm. The tip of the probe was attached manually to the site of drilling for cochlear implantation. Blood flow was measured before, during, and after the cochlear bony wall had been opened, as demonstrated in Fig 2. For drilling, we used a Skeeter drill (Medtronic Xomed, Jacksonville, Florida) with an outer diameter of 0.8 mm.

Figure 2A shows the tip of the laser-Doppler probe attached to the mucous membrane of the promontory before drilling commenced. Figure 2B shows the placement of the tip of the probe when the mucous membrane of the promontory had been removed

by the drilling. Figure 2C shows the placement of the tip of the probe when half of the bony wall had been drilled. Figure 2D shows the tip of the probe attached to the lateral membrane of the scala tympani. Figure 2E shows the placement after the bony wall had been opened, with the tip of the probe inserted into the perilymphatic space. It took less than 1 minute to drill through half of the bone. The drill speed was about 12,000 rpm. We used saline solution several times to cool the bone during the drilling.

RESULTS

The Table gives the values of the laser-Doppler output measured at the positions shown in Fig 2. The laser-Doppler output was largest when the tip of the probe was attached to the mucous membrane of the promontory (analysis of variance, $p < .01$). As shown in the Table, measurements could not be done at some positions, when it was considered that the drilling proceeded too deep for the measurement. An example of the recording is demonstrated in Fig 3. Figure 3 shows that the laser-Doppler output became stable within several seconds after the placement of the probe. It took about 10 minutes for the evaluation of blood flow, including 5 measurements (Fig 2). Figure 3 also shows a tendency for pulsatory movement of the output to be wide when the output was large.

The output was confirmed in all situations and cases, except in 2 patients, for whom the output was zero when measured at the position shown in Fig 2D. Even in the patient with narrow internal auditory canals, output was confirmed in all situations, as shown in the Table. There was no significant difference among the values of the laser-Doppler output measured at the positions shown in Fig 2B-E. A correlation was observed between the values of the laser-Doppler output measured when the tip of the probe was attached to the mucous membrane of the promontory and those measured when the tip of the probe was inserted into the perilymph ($r = 0.78$, $p < .01$). However, there was no significant correlation between the values of the laser-Doppler output mea-

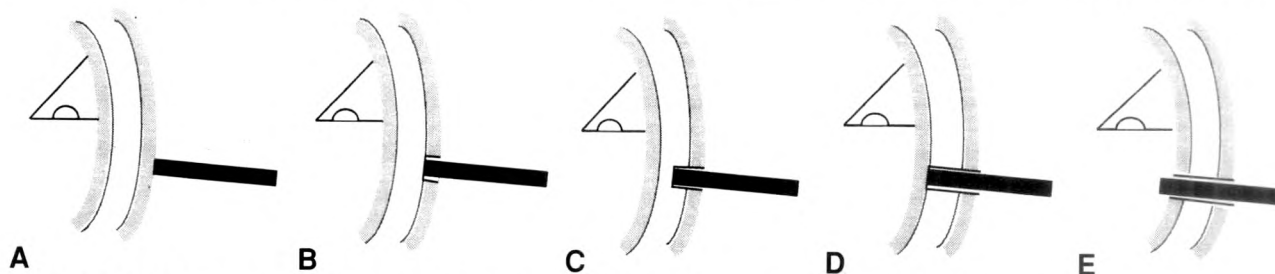


Fig 2. Blood flow measurements in ears of patients receiving cochlear implants. **A)** Tip of probe is on mucous membrane of promontory. **B)** Mucous membrane of promontory has been removed. **C)** Half of bony wall has been drilled. **D)** Bony wall has been drilled down to mucous membrane surrounding perilymph. **E)** Bony wall has been opened, and tip of probe has been inserted into perilymphatic space.

LASER-DOPPLER OUTPUT FROM EARS RECEIVING COCHLEAR IMPLANTS

Patient No.	Age (y)	Sex	Hearing Loss	Output at A	Output at B	Output at C	Output at D	Output at E
1	16	F	Hereditary	5.3	2.9	4.0	1.9	2.7
2	3	F	Waardenburg's syndrome	44.8	4.0	2.3	0.0	5.0
3	45	F	IPSNHL	4.2	1.4	2.4	0.0	1.1
4	2	F	Congenital	27.8	1.3	NM	2.2	4.6
5	3	M	Narrow IAC	3.8	2.5	0.4	1.8	1.4
6	3	M	Congenital	2.2	NM	1.0	0.2	0.7
7	55	M	IPSNHL	1.0	0.6	NM	NM	0.4
8	3	M	Congenital	5.9	3.0	1.1	NM	2.7
9	2	M	Congenital	22.8	2.1	2.2	NM	2.5
10	26	F	IPSNHL	6.8	NM	1.5	NM	4.0
11	2	M	Congenital	4.5	1.2	1.3	0.6	0.8
12	53	M	Sudden deafness	15.6	1.9	NM	7.0	2.3

Outputs (arbitrary units) at A, B, C, D, and E represent those measured at positions shown in Fig 2A-E, respectively.

IPSNHL — idiopathic progressive sensorineural hearing loss, NM — not measured, IAC — internal auditory canal.

sured when the tip of the probe was attached to the mucous membrane of the promontory and those measured at the positions shown in Fig 2B-D.

There was no correlation between the laser-Doppler output and blood pressure. When the patients were analyzed as 2 groups — adults (16 years or older) and children (3 years or younger) — the laser-Doppler output was not significantly different for any measurement taken in the positions shown in Fig 2.

DISCUSSION

After measuring CBF by using laser-Doppler flowmetry, Miller et al⁹ reported that normal mucosa in humans does not contribute significantly to the laser-Doppler output. Nakashima et al¹⁰ reported that removal of the middle ear mucosa under the probe tip lowered the laser-Doppler output to 50% to 80%. In the present study, removal of the middle ear mucosa lowered the laser-Doppler output to 33% of the average of its previous level. This large reduction in output may suggest a disturbance of CBF in ears receiving cochlear implants.

Because we opened the bony wall by using a drill,

blood flow in the bony wall or blood flow in the perilymphatic lateral membrane adjacent to the drilled part may be disturbed by the mechanical or thermal effect of the drilling. However, we consider that the laser-Doppler output measured when the tip of the probe was inserted into the perilymphatic space reflected CBF without the mechanical or thermal effect of the drilling, because the output reflected blood flow in the area illuminated by the laser light through the transparent perilymph, in which it is distant to the drilled part. In measuring blood flow where the measurement portion is not transparent, blood flow in the portion within about 1 mm from the tip of the probe can be measured with this laser probe.

In this study, we did not observe bleeding when the bony wall was opened (from Fig 2B to Fig 2E). This finding is in contrast to the fact that bleeding always occurs when the bony wall is opened in normal guinea pigs. Actually, using the same probe in guinea pigs, we confirmed that the laser-Doppler output measured when the middle ear mucosa was intact was not significantly different from that measured when the probe tip was inserted into the perilymph. In a case with a narrow internal auditory ca-

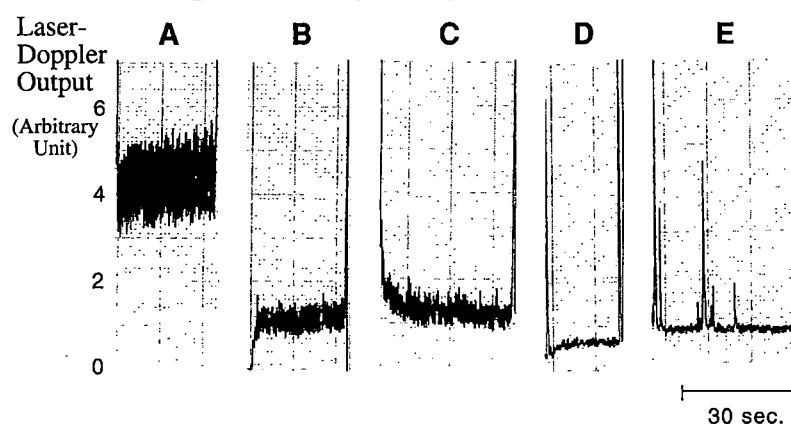


Fig 3. (Case 11) Example of recording of laser-Doppler output measured at positions in Fig 2A-E. Each recording was made from placement to removal of probe.

nal, reduction of CBF was anticipated, because the inner ear artery runs through the internal auditory canal. However, the laser-Doppler output in this case was not significantly different from those in the other cases. These findings may also suggest disturbance of CBF in patients who receive cochlear implants.

Fibrous or bony occlusion of the perilymphatic space, which occasionally makes cochlear implantation difficult, may be associated with impairment of CBF. Perlman et al¹⁹ described that experimental obstruction of the labyrinthine artery was rapidly followed by severe degenerative changes and ossification in the cochlea. Human temporal bones, after surgical removal of acoustic tumor or sudden sensorineural hearing impairment, also revealed cochlear ossification that suggested a vascular cause of hearing loss.²⁰ Cochlear ossification is a common occurrence in postmeningitic deaf patients.^{21,22} However, whether the postmeningitic cochlear ossification is associated with the disturbance of CBF is not clear

at present.

Because laser-Doppler flowmetry provides only relative values, it is difficult to compare results across patients. However, laser-Doppler flowmetry is superior for examining relative changes due to various stimuli. It has been shown that direct electrical stimulation of the cochlea elevates CBF.^{1,9,23} As electrical stimulation to the cochlea has been recently applied to observe neural responsiveness during a cochlear implant procedure (eg, as neural response telemetry), we believe that observation of the laser-Doppler response to such electrical stimulation may provide further information about CBF.

In conclusion, laser-Doppler flowmetry is both relatively safe and useful for measuring blood flow in the ears during cochlear implantation procedures. The measurement may be useful in, among other things, investigating the cause of sensorineural hearing loss and investigating the relationship between ossification and disturbance of blood flow.

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BACTERIOLOGY OF ACUTE AND CHRONIC SPHENOID SINUSITIS

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Aspirates of 16 acutely infected and 7 chronically infected sphenoid sinuses were processed for aerobic and anaerobic bacteria. A total of 29 isolates were recovered from the 16 cases of acute sphenoid sinusitis (1.8 per specimen): 22 aerobic and facultative (1.4 per specimen), and 7 anaerobic (0.4 per specimen). Aerobic and facultative organisms alone were recovered in 10 specimens (62%), anaerobes alone were isolated in 3 (19%), and mixed aerobic and anaerobic bacteria were recovered in 3 (19%). The predominant aerobic and facultative species were *Staphylococcus aureus* (9 isolates), *Streptococcus* spp (9), and *Haemophilus influenzae* (2). A total of 28 isolates were recovered from the 7 cases of chronic sphenoid sinusitis (4.0 per specimen): 11 aerobic and facultative (1.6 per specimen) and 17 anaerobic (2.4 per specimen). Aerobic and facultative organisms alone were recovered in 1 instance (14%), anaerobes alone in 3 instances (43%), and mixed aerobes and anaerobes in 3 instances (43%). The predominant aerobic bacteria were gram-negative bacilli (*Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*; 1 each). The predominant anaerobes included *Peptostreptococcus* spp (4 isolates), *Prevotella* spp (5), and *Fusobacterium* spp (4). These findings illustrate the unique microbiology of acute and chronic sphenoid sinusitis.

KEY WORDS — anaerobe, beta-lactamase, *Fusobacterium* spp, *Prevotella* spp, sphenoid sinusitis, *Staphylococcus aureus*.

INTRODUCTION

Sphenoid sinusitis is a rare but potentially devastating infection. In contrast to maxillary sinusitis, the microbiology of sphenoid sinusitis is not well established, and only a few reports have documented the organisms isolated.¹⁻³ The role of anaerobic bacteria in this infection has not been well studied either, although their recovery has been recorded in a few cases.¹⁻³

This report describes the author's personal experience during a 25-year period of studying the aerobic and anaerobic microbiology of sphenoid sinusitis.

PATIENTS AND METHODS

The 23 patients included in the report were studied by the author between June 1975 and June 2000. Excluded were 6 additional patients whose sphenoid sinusitis showed no bacterial growth. They were seen in the following hospitals: University of California Medical Center and County Medical Center in Los Angeles, California; Children's Hospital National Medical Center in Washington, DC; and Naval Hospital in Bethesda, Maryland.

The patients' ages ranged from 15 to 69 years (mean, 42 years 7 months), and 13 were male. Three patients were children (younger than 18 years old). Included in the study were 16 patients with acute and 7 with chronic sinusitis. Antimicrobial therapy was administered to 16 patients (70%) in the month before sample collection. These included 11 patients

with acute and 5 with chronic sinusitis.

Only patients who fulfilled the following criteria were included in the study: typical clinical symptoms of sinusitis (headache, fever, nasal drainage, etc), positive radiographic findings, positive sphenoid bacterial cultures, and biopsy specimens demonstrating acute or chronic inflammation of the sinus mucosal lining, or clinical and radiologic findings compatible with sphenoid sinusitis followed by clinical and radiologic improvement after surgery and treatment with antibiotics.

The sinusitis was considered acute if the duration of symptoms was less than 1 month; chronic symptoms were those that persisted for more than 1 month.

Sphenoid surgery was performed by an external ethmoidal approach in 14 patients or by an intranasal endoscopic approach in 9. The secretions were mucopurulent in 17 instances (11 acute and 6 chronic) and opaque in 6 (5 acute and 1 chronic). A foul smell was noted in 11 instances: 6 chronic and 5 acute.

The specimens were obtained during surgery after the sinuses were opened in 13 cases, and through a puncture before the sinuses were opened in 10. Strict asepsis was used to avoid any contamination, and the specimens were transported to the laboratory in a syringe sealed with a rubber stopper after evacuation of the air or in an anaerobic transport tube (Port-A-Cul, Baltimore Biological Laboratories, Cockeysville, Maryland). The time between the collection of materials and inoculation of the specimen was generally less than 60 minutes for syringes and

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ORGANISMS ISOLATED FROM 23 ASPIRATES OF SPHENOID SINUSITIS

Bacteria	Acute Sinusitis (n = 16)	Chronic Sinusitis (n = 7)	Total (n = 23)
Aerobic bacteria			
<i>α</i> -Hemolytic streptococci	3	2	5
Microaerophilic streptococci	2	2	4
<i>Streptococcus pneumoniae</i>	1		1
<i>Streptococcus pyogenes</i>	1		1
Group F streptococci	2		2
<i>Staphylococcus aureus</i>	9 (9)	1 (1)	10 (10)
<i>Staphylococcus epidermidis</i>	1	2 (1)	3 (1)
<i>Klebsiella pneumoniae</i>		1 (1)	1 (1)
<i>Escherichia coli</i>		1	1
<i>Pseudomonas aeruginosa</i>	1 (1)	1 (1)	2 (2)
<i>Haemophilus influenzae</i>	2 (1)	1 (1)	3 (2)
Subtotal	22 (11)	11 (5)	33 (16)
Anaerobic bacteria			
<i>Peptostreptococcus</i> spp	3	4	7
<i>Propionibacterium acnes</i>	2	2	4
<i>Fusobacterium</i> spp		1 (1)	1 (1)
<i>Fusobacterium nucleatum</i>	1	3 (2)	4 (2)
<i>Bacteroides</i> spp		1 (1)	1 (1)
<i>Prevotella melaninogenica</i>	1	2 (1)	3 (1)
<i>Prevotella oralis</i>		1 (1)	1 (1)
<i>Prevotella intermedia</i>		2 (1)	2 (1)
<i>Porphyromonas asaccharolytica</i>		1	1
Subtotal	7 (0)	17 (7)	24 (7)
Total	29 (11)	28 (12)	57 (23)

Numbers within parentheses indicate β -lactamase producers.

less than 3 hours for the anaerobic transport tube.

Specimens were inoculated onto 5% sheep's blood, chocolate agar, and MacConkey agar plates for aerobic and facultative organisms. The plates were incubated at 37°C aerobically (MacConkey) or under 5% carbon dioxide (5% sheep's blood and chocolate) and examined at 24 and 48 hours. For anaerobes, the material was plated onto prereduced vitamin-K1-enriched *Brucella* blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, an anaerobic blood plate containing colistin and nalidixic acid, and an enriched thioglycolate broth (containing hemin and vitamin K1).⁴ The anaerobic plates were incubated in anaerobic jars (GasPak jars, Baltimore Biological Laboratories) and examined at 48 and 96 hours.

Anaerobes were identified by techniques described previously.⁴ Aerobic bacteria were identified by conventional methods.⁵ β -Lactamase activity was determined by use of the chromogenic cephalosporin analog 87/312 method.⁶ Blood cultures for aerobic and anaerobic bacteria were obtained from 17 patients before surgery.

RESULTS

Acute Sphenoid Sinusitis. A total of 29 isolates were recovered from the 16 cases (1.8 per specimen): 22 aerobic and facultative (1.4 per specimen) and 7 anaerobic (0.4 per specimen). The number of isolates per specimen varied from 1 to 4. Aerobic and facultative organisms alone were recovered in 10 specimens (62%), anaerobes alone were isolated in 3 (19%), and mixed aerobic and anaerobic bacteria were recovered in 3 (19%). The predominant aerobic and facultative organisms were *Staphylococcus aureus* (9 specimens), *Streptococcus* spp (9), and *Haemophilus influenzae* (2; see Table). The predominant anaerobic bacteria were *Peptostreptococcus* spp (3) and *Propionibacterium acnes* (2). Eleven β -lactamase-producing bacteria (BLPB) were recovered from 10 specimens (50%). The predominant one was *S. aureus*.

Chronic Sphenoid Sinusitis. A total of 28 isolates were recovered from the 7 cases (4.0 per specimen): 11 aerobic and facultative (1.6 per specimen) and 17 anaerobic (2.4 per specimen). The number of isolates varied from 2 to 6. Aerobic and facultative organisms were recovered in 1 instance (14%), anaerobes alone in 3 instances (43%), and mixed aerobic and anaerobic bacteria in 3 instances (43%). The predominant aerobic bacteria were gram-negative bacilli (*Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*; see Table). The predominant anaerobes included *Peptostreptococcus* spp (4), *Fusobacterium* spp (4), and *Prevotella* spp (5). Twelve BLPB were recovered from 6 patients (86%). These included the *S. aureus* isolate, 3 of the 4 isolates of *Fusobacterium* spp (75%), and 3 of the 5 isolates of *Prevotella* spp (60%).

Concomitant medical problems included hypertension (4 cases), asthma (3 cases), diabetes (3 cases), oral steroid use (2 cases), deep-sea diving (2 cases), pituitary malignancy (1 case), and trauma of the skull (1 case). No correlation was found between these conditions and the microbiological findings.

Organisms similar to those recovered in the sinuses were found in the blood in 5 instances. These included *S. aureus* (3 cases), *Fusobacterium nucleatum* (1 case), and *Prevotella melaninogenica* (1 case).

DISCUSSION

This study demonstrates the microbiological features of acute and chronic sphenoid sinusitis. Unlike in acute maxillary sinusitis, in which *Streptococcus pneumoniae* predominates, *S. aureus* is the most common isolate in acute sphenoid sinusitis. Similar findings were also observed by other investigators.¹⁻³

Lew et al¹ recovered *S aureus* in 5 of 13 patients (38%) with acute sphenoid sinusitis. The present study revealed that chronic sphenoid sinusitis involves more organisms per specimen as compared to acute infection, and that the most common isolates in chronic infection were anaerobic bacteria. These were mainly *Peptostreptococcus* spp, *Fusobacterium* spp, pigmented *Prevotella*, and *Porphyromonas* spp — all members of the oropharyngeal flora. Similarly to Lew et al, aerobic gram-negative bacilli (including *K pneumoniae*, *E coli*, and *P aeruginosa*) were also isolated in the present study in chronic sphenoid sinusitis. The high recovery rate of anaerobic bacteria in chronic sphenoid sinusitis is similar to the findings in chronic maxillary sinusitis, in which these organisms also predominate.⁷⁻⁹

The frequent involvement of anaerobes in chronic sphenoid sinusitis probably could be related to the poor drainage and increased intranasal pressure (noted in the maxillary sinus) that develops during inflammation.¹⁰ This can reduce the oxygen tension in the inflamed sinus¹¹ by decreasing the mucosal blood flow¹² and depressing the ciliary action.¹³ The lowering of the oxygen content and pH of the sinus cavity supports the growth of anaerobic organisms by providing them with an optimal oxidation-reduction potential.¹³

In 14 of 23 patients (61%), BLPB were isolated. The recovery of BLPB is not surprising, because al-

most three quarters of the patients in this study received antimicrobial agents, including the β -lactams, within the past 3 months, which might have selected for these organisms.

Surgical drainage is essential in most cases, and an initial empiric broad-spectrum antimicrobial coverage is required. However, the unique microbiology of acute and chronic sphenoid sinusitis, and the recovery of BLPB in about two thirds of the specimens, requires adjusting an initial empiric therapy to a specific one.

Empiric broad-spectrum coverage with a combination of a penicillin and a β -lactamase inhibitor, or clindamycin (effective against anaerobes and *S aureus*), plus an antimicrobial effective against aerobic gram-negative rods (ie, an aminoglycoside, ceftazidime, or a quinolone in adults) or a carbapenem (ie, imipenem), may be warranted initially. Additional coverage for *S aureus* may be achieved by use of a β -lactam-resistant penicillin (eg, dicloxacillin), vancomycin, or linezolid. Specific antimicrobial therapy can be chosen after culture results are obtained.

Prospective studies are warranted to elucidate the role of anaerobic bacteria in acute and chronic sphenoid sinusitis. It is, however, recommended that specimens from infected sphenoid sinuses be cultured for both aerobic and anaerobic bacteria, as well as fungi, so that antimicrobial therapy can be appropriate.

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SENSITIVITY AND SPECIFICITY OF INTRAOPERATIVE RECURRENT LARYNGEAL NERVE STIMULATION IN PREDICTING POSTOPERATIVE NERVE PARALYSIS

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Bilateral recurrent laryngeal nerve (RLN) paralysis after thyroidectomy is infrequent, but serious when it occurs. Intraoperative knowledge of the status of the nerve after dissection could potentially provide the surgeon with important decision-making information. The current study examines the sensitivity and specificity of intraoperative stimulation of the RLN during thyroid surgery for predicting postoperative RLN deficits. Eighty-one RLNs in 55 patients were identified to be at risk of injury during thyroidectomy or parathyroidectomy performed between January 1998 and February 2000. Intraoperative determination of RLN function was evaluated with a disposable nerve stimulator (Xomed, Jacksonville, Florida) set at 0.5 mA. Injury was assessed by palpating for a contraction of the posterior cricoarytenoid muscle while the stimulus was applied. Postoperative assessment of RLN integrity was determined by using indirect or direct laryngoscopy to visualize vocal fold mobility. Nine RLNs failed to elicit a posterior cricoarytenoid contraction after nerve stimulation, and 4 RLNs were determined to be deficient in the postoperative evaluation. The calculated sensitivity and specificity were 75% and 92.2% with a positive predictive value of 33.3% and negative predictive value of 98.6%. The RLN injury rate was 4.94%. We conclude that intraoperative RLN stimulation is a relatively safe and useful method of determining what RLN function will be after thyroid or parathyroid surgery.

KEY WORDS — nerve paralysis, thyroid surgery.

INTRODUCTION

Iatrogenic injury of the recurrent laryngeal nerve (RLN) is a cause of morbidity associated with surgical procedures of the head and neck. In particular, surgery of the thyroid and parathyroid glands carries a substantial risk of injury to the RLN because of the structures' intimate anatomic relationship. The reported incidence of RLN injury in thyroid gland surgery varies widely, from 0.4% in primary operations to 12.5% in secondary operations.¹⁻⁹ Several factors that increase the incidence of RLN injury include underlying thyroid disease, malignancy, prior irradiation, extent of surgical resection, previous thyroid or parathyroid surgery, anatomic variability or anomalies, and failure to identify the nerve.^{3,4} Despite the variable incidence of RLN injury, intraoperative identification of the RLN has been a concern of surgeons and has become an established and integral part of thyroid surgery since Lahey's 1938 article.^{5,10}

In addition to visual identification of the RLN during surgery, electrophysiological means of identifying and assessing RLN function have become available in recent years. Several studies present a method of RLN monitoring via direct electrical RLN stimulation and measurement of evoked electromyographic responses with either an endotracheal tube with integrated electrodes or postcricoid laryngeal surface

electrodes.^{3,5,6} Others have used a method of direct stimulation of the RLN with a disposable nerve stimulator and palpation of a response of the laryngeal musculature.⁷⁻⁹

The current study examines the sensitivity and specificity of intraoperative stimulation of the RLN during thyroid surgery for predicting postoperative RLN deficits.

METHODS

Data were collected retrospectively from 60 patients who underwent thyroid or parathyroid surgery between January 1998 and February 2000. Each patient underwent preoperative assessment by either fiberoptic or indirect laryngoscopy to assess vocal fold mobility. During the surgical procedures, an attempt was made to isolate and expose each RLN at risk. At the conclusion of each procedure, the identified RLN was stimulated proximal to its entrance into the larynx with a disposable Xomed nerve stimulator (Xomed, Jacksonville, Florida) set at 0.5 mA.

Intraoperative determination of RLN injury was assessed by digital palpation for a contraction of the posterior cricoarytenoid (PCA) muscle while the stimulus was applied. In instances in which no contraction was palpated, a second 0.5-mA stimulus was

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TABLE 1. RESULTS OF INTRAOPERATIVE TESTING OF RLN AND POSTOPERATIVE OBSERVATIONS OF VOCAL FOLD MOBILITY

<i>Results of Intraoperative Stimulation of RLN</i>	<i>Results of Postoperative RLN Examination</i>	
	<i>Vocal Fold Paralysis</i>	<i>Normal Vocal Fold Mobility</i>
No contraction (positive)	3	6
Contraction (negative)	1	71

RLN — recurrent laryngeal nerve.

applied. A positive test result (ie, injured nerve) for RLN injury was defined as “no contraction,” whereas a negative result (ie, noninjured nerve) was defined as a “palpable contraction.” Postoperative assessment of the integrity of each RLN was performed within 1 week of the procedure by fiberoptic or indirect laryngoscopy to visualize the mobility of the vocal folds. All examinations were performed by otolaryngology residents proficient in laryngeal examination. The sensitivity, specificity, positive predictive value, negative predictive value, and RLN injury rate were calculated from the collected data.

Additionally, in those patients in whom postoperative RLN palsies were noted, subsequent patient records were reviewed to determine whether the RLN injury was temporary or permanent.

RESULTS

A total of 81 RLNs were identified as “at risk” of injury in 55 of the 60 patients who underwent thyroidectomy or parathyroidectomy during the specified time period. Five patients in whom RLNs were not identified during operation were eliminated from the study, and 2 RLNs with preexisting deficits were not included in the data.

Intraoperative RLN stimulation failed to elicit a PCA contraction in 9 RLNs (Table 1). Of the 9 positive results, 3 RLNs were subsequently determined to be injured in the immediate postoperative period by fiberoptic examination as evidenced by vocal fold weakness, and the remaining 6 RLNs were shown to be intact after operation. Intraoperative stimulation of 72 RLNs did elicit PCA contractions (negative result). One RLN with a negative test result for injury was found to be nonfunctional in the immediate postoperative period, but the problem subsequently resolved.

Seventy-five percent of RLNs with injuries failed to demonstrate intraoperative contraction of the PCA muscle (sensitivity, 75.0%). Ninety-two percent of RLNs without injury demonstrated contraction of the PCA muscle (specificity, 92.2%). The positive predictive value and negative predictive value were calcu-

TABLE 2. SENSITIVITY, SPECIFICITY, AND POSITIVE AND NEGATIVE PREDICTIVE VALUES OF INTRAOPERATIVE STIMULATION OF RLN

Sensitivity	75.0%
Specificity	92.2%
Positive predictive value	33.3%
Negative predictive value	98.6%

lated to be 33.3% and 98.6%, respectively (Table 2).

Of the 4 patients in whom RLNs were found to be injured in the immediate postoperative period, 3 RLN injuries resolved upon reexamination at a later date, and 1 patient was lost to follow-up.

DISCUSSION

Iatrogenic injury of the RLN is a serious complication of head and neck surgery. Mechanisms of permanent RLN injury have been noted to include transection, stretch injury, crush injury, electrical injury, thermal injury, and ischemia.³ Temporary RLN paralysis may occur as a result of edema, inflammation, ligature entrapment, or minor trauma to the nerve itself caused by manipulation.^{2,4} Regardless of the duration of injury, deficits in RLN function have dire consequences for the patient. Common symptoms or complications of unilateral RLN paralysis include dysphonia, susceptibility to aspiration pneumonia, cardiac stress, and ventilatory disturbances in the form of airflow resistance.^{2,6} Bilateral paralysis can be life-threatening because of acute airway obstruction.⁵ For this reason, some surgeons will perform direct laryngoscopy at the completion of the surgery.

Whereas previous studies have advocated intraoperative nerve stimulation as a means to identify and localize the RLN, the focus of the current study was to examine the value of determining RLN integrity in assessing and identifying patients who are likely to have postoperative deficits with subsequent complications. A positive test result for RLN injury could allow the surgeon to search for the cause of the nerve deficit and identify potentially reversible causes of temporary RLN deficits. Similarly, positive test results for RLN injury seen during the procedure could prompt the surgeon to consider equally effective alternative treatment options (eg, lobectomy, subtotal thyroidectomy, near-total thyroidectomy) or alternate postoperative monitoring regimens.

The data show that intraoperative stimulation of the RLN is a useful test in determining the postoperative condition of nerve function; an RLN determined to be functioning at the time of the procedure is likely to be intact, given the high specificity rate of 92.2% and the negative predictive value of 98.6%. Although the calculated sensitivity of 75% indicates

that a positive test result may not accurately depict the viability of the RLN in the postoperative period, a positive result does alert the surgeon to patients with possible RLN dysfunction who should be monitored closely during the postoperative period.

Possible reasons for the noted false-positive results include inaccurate nerve stimulator placement, inadequate stimulus amplitude, and a failure of the physician to palpate a muscular contraction. A false-

negative result could be caused by nerve stimulation proximal to the site of injury or an erroneous sensation palpated by the surgeon.

In conclusion, the current study outlines a useful, practical, and inexpensive method of intraoperative assessment of RLN function. However, this method of RLN monitoring should not preclude or replace a thorough head and neck evaluation to assess the integrity of the RLN after operation.

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ELECTROPHYSIOLOGICAL FINDINGS IN TWO BILATERAL COCHLEAR IMPLANT CASES: DOES THE DURATION OF DEAFNESS AFFECT ELECTRICALLY EVOKED AUDITORY BRAIN STEM RESPONSES?

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Bilateral cochlear implantation provides an interesting model for in vivo study of the effect of long-term profound deafness on neural transmission. We present electrophysiological observations on 2 patients implanted with the MXM Binaural Digisonic Convex system. This uncommon design consists of 2 electrode arrays placed bilaterally into the scala tympani and controlled by a single speech processor. In both patients, the duration of deafness before cochlear implantation clearly differed from one ear to the other. Electrically evoked auditory brain stem responses (EABRs) were measured and the EABRs from the ear with the longer deafness duration showed a lengthening of wave V latency. In 1 patient, recordings from this ear also showed a lack of reproducibility of wave III. The data suggest that neural responsiveness in the peripheral and intermediate auditory pathways is adversely affected by deafness duration. Poor EABRs on one ear possibly result from the total duration of deafness in this ear and/or compensation by the other ear.

KEY WORDS — bilateral cochlear implantation, deafness duration, electrically evoked auditory brain stem response.

INTRODUCTION

To our best knowledge, the electrically evoked auditory brain stem response (EABR) in bilateral cochlear implantation has so far only been reported in a patient equipped with the Ineraid cochlear implant involving a device with a percutaneous plug, and who had undergone 2-step surgery.¹ Recently, we had the opportunity to study EABR in 2 patients equipped with the Binaural Digisonic Convex cochlear implant (MXM Laboratory, Vallauris, France). In this design, 2 electrode arrays are placed bilaterally into the scala tympani during a single surgical session and are controlled by a single receiver placed on one side. As a result, such a device provides electrical stimulation similar to binaural acoustic stimulation. In both of the cases reported here, the duration of deafness (Δt_D) clearly differed from one ear to the other, before the implantation procedure. Thus, this case study provided an opportunity to investigate the influence of Δt_D on EABR.

MATERIAL AND METHODS

Binaural Digisonic Convex Cochlear Implant. The

MXM Binaural Digisonic Convex is a multielectrode transcutaneous cochlear implant. The implanted part of the device consists of a single coil receiver and 15 0.5-mm-diameter electrodes (numbered 1 to 15). The electrodes are shared between 2 arrays, 1 for each ear. Electrodes 1 and 2 are the most basal, and electrodes 14 and 15 are the most apical. The odd-numbered electrodes (1, 3, 5, 7, 9, 11, 13, and 15) are distributed in the right array, and the even-numbered electrodes (2, 4, 6, 8, 10, 12, and 14) in the left array. The whole system is driven by 1 single speech processor, which allows both sides to be activated simultaneously. On each side, the stimulation mode used is the "common ground" mode, in which 1 electrode at a time is activated, all the others being grounded.

Both patients underwent bilateral 1-step surgery, with full insertion of electrode arrays into the scala tympani. The insertion was symmetric (Fig 1). At 2 months after surgery, each patient had fully active electrode arrays on both sides.

EABR Recordings. The EABRs were recorded between a positive silver-silver chloride electrode centrally placed on the forehead and a negative one po-

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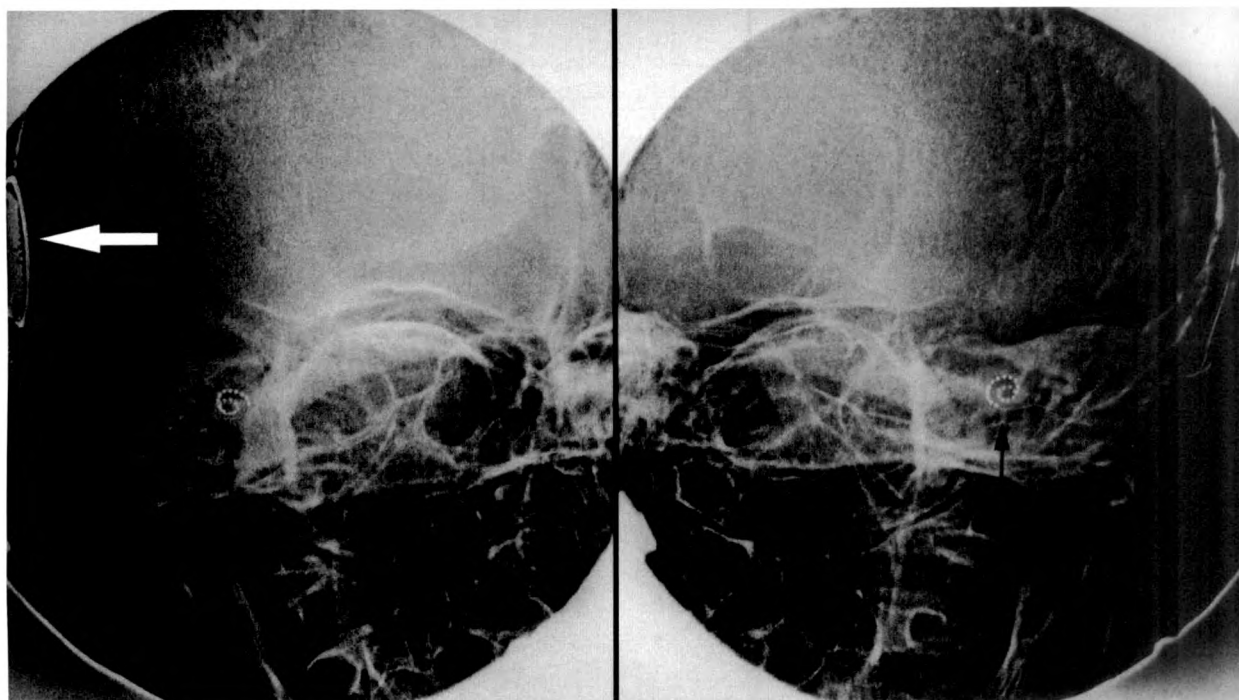


Fig 1. (Subject 1) Radiologic images show symmetrically placed electrode arrays in scala tympani (black arrows). Receiver is positioned on right side (white arrow).

sitioned under the chin. A further electrode attached to the forehead served as a ground.^{2,3}

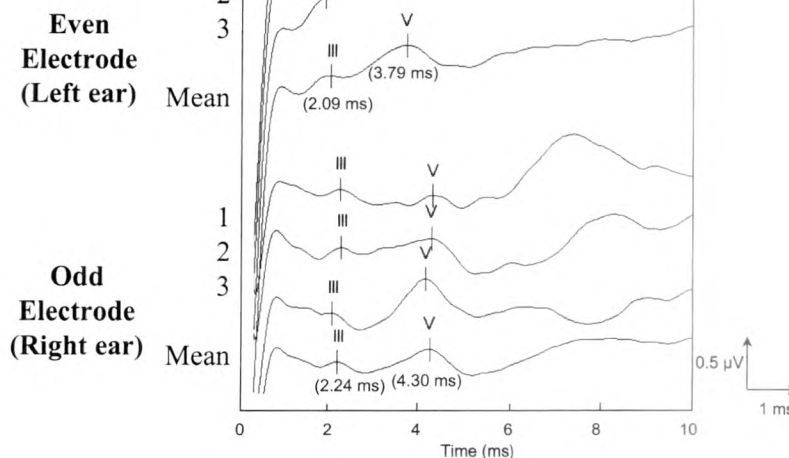
Electrical stimuli were generated by the manufacturer's interface device (Digistim system, MXM Laboratory) connected to a computer via a serial port. This system also triggered the evoked-potential measurement device (Centor system, Racine-Alvar, Bordeaux, France). In both subjects, EABRs were measured in response to stimuli in 3 bilateral pairs of electrodes: 1 basal (pair 2-3), 1 midrange (pair 8-9), and 1 apical (pair 12-13). A pair consisted of elec-

trodes positioned symmetrically, each in one cochlea. For each pair of electrodes, a comparison was made between EABRs obtained from either side.

The programming setup used arbitrary units rather than pulse-duration units to represent stimulus levels. The EABRs were obtained for each tested electrode at a stimulation level corresponding to 70% of the dynamic range defined during the implant fitting session. The stimulation rate was set at 54 Hz.

The responses were filtered with a 1.6- to 1,600-

Fig 2. (Subject 1) Electrophysiological traces obtained for midrange pair of electrodes (8-9) at 6-month postoperative interval. For both electrodes, 3 blocks of 1,000 sweeps are presented (1, 2, 3), followed by mean electrically evoked auditory brain stem response (EABR) trace. EABR peaks are identified, for each curve, as zero crossing points in second derivative of signal. Wave latencies on mean traces are indicated in parentheses.



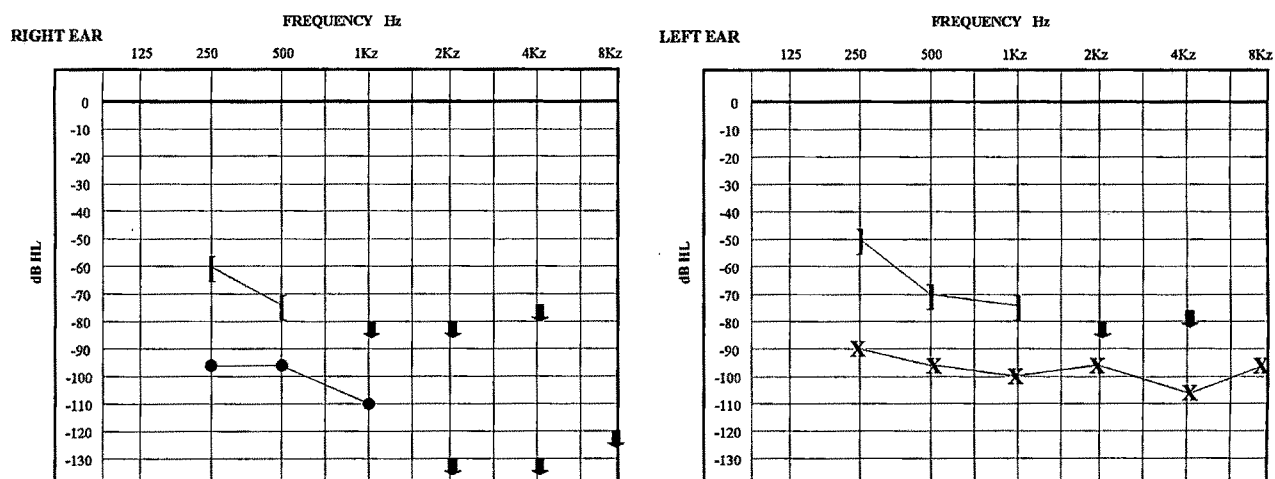


Fig 3. (Subject 1) Pure tone audiogram obtained 1 month before bilateral implantation.

Hz analog bandpass filter and with the amplifier sensitivity set to 100 μ V. An analysis time of 10 ms was used with a sampling rate of 25 kHz. For each tested electrode, the initial averaging process involved 1,000

sweeps. To verify the reproducibility of the EABR peaks obtained, we collected 3 averages of 1,000 sweeps per stimulus configuration. The 3 blocks were then summed and averaged to provide the mean trace. The wave latencies were determined from an algorithm based on the detection of peaks and inflection points in the first derivative of the signal. According to this algorithm, adapted from Gabriel et al,⁴ detection of zero crossings in the second derivative of the averaged potentials can be used to identify waves III and V in EABR recordings. The wave latencies measured on the mean traces were used for comparison between ears. Figure 2 shows typical EABR recordings obtained by this method. Further details about the EABR recording and processing methods may be found in previous articles by our team.^{3,5,6}

CASE REPORTS

Case 1. A 52-year-old man had developed progressive degenerative hearing loss resulting in profound right-sided deafness when he was 12 years old. His left-side hearing began to fail when he was a young adult. At the age of 45 years, the deterioration in his hearing function led to a severe left-sided deafness, and he lost the ability to communicate at conversational levels. He was consequently fitted with left ear amplification. The left auditory performance continued to deteriorate afterward, especially during the year that led up to binaural implantation, resulting in bilateral profound deafness (Fig 3). Thus, the duration of profound deafness, Δt_D , could be estimated as 40 years for the right ear and as 1 year for the left.

The EABRs were recorded at 3 and 6 months after surgery. Measurements from electrodes with symmetric intracochlear locations (2-3, 8-9, and 12-13) were paired. Figure 4 shows the patient's EABRs obtained 3 months after surgery in apical, midrange, and basal pairs. Each curve represents the result of

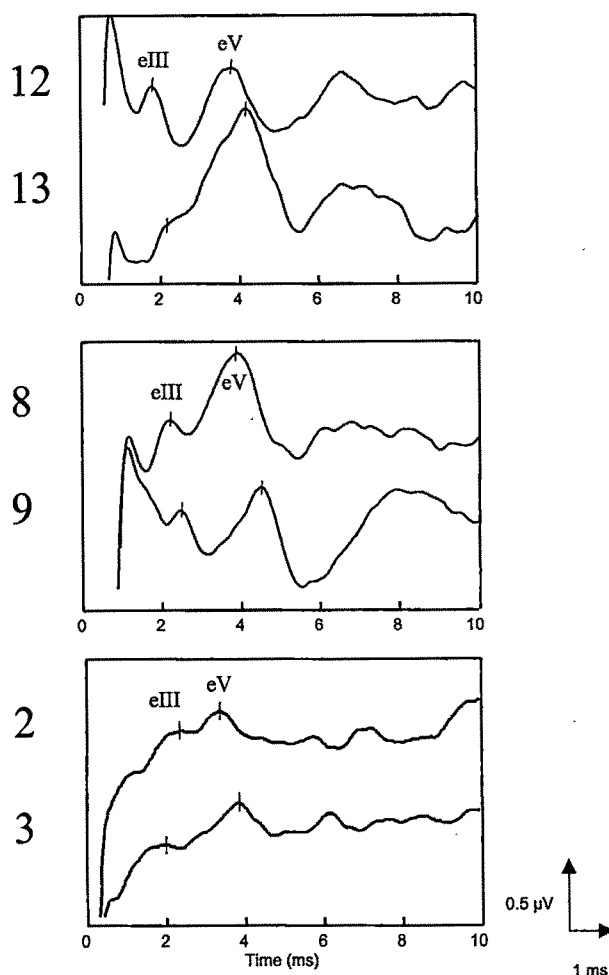


Fig 4. (Subject 1) EABRs obtained at postoperative interval of 3 months for tested apical (12-13), midrange (8-9), and basal (2-3) electrodes. For each pair of electrodes, upper curve corresponds to recordings from ear with shorter deafness duration.

ELECTRICALLY EVOKED AUDITORY BRAIN STEM RESPONSE WAVE LATENCIES

Pair of Tested Electrodes	Interval of Testing (mo)	Odd Electrode		Even Electrode		Interaural Shift in Wave Latencies (ms)	
		LIII	LV	LIII	LV	dLIII	dLV
Subject 1							
Apical	3	2.17	4.17	1.98	3.90	0.19	0.26
Apical	6	1.88	4.75	2.05	4.31	−0.17	0.44
Midrange	3	2.36	4.51	2.29	3.96	0.07	0.54
Midrange	6	2.24	4.30	2.09	3.79	0.14	0.50
Basal	3	2.08	3.99	2.31	3.56	−0.22	0.44
Basal	6	2.35	4.15	2.27	3.72	0.07	0.42
Mean ± SD		2.18 ± 0.18	4.31 ± 0.28	2.17 ± 0.14	3.88 ± 0.26	0.01 ± 0.17	0.44 ± 0.09
Subject 2							
Apical	6	No wave III	5.24	2.05	3.95		1.29
Apical	9	2.80	5.40	2.04	4.24	0.76	1.16
Midrange	6	No wave III	5.56	1.96	3.76		1.80
Midrange	9	No wave III	5.56	2.12	3.96		1.60
Mean ± SD			5.44 ± 0.15	2.04 ± 0.07	3.98 ± 0.20		1.46 ± 0.29
Data are in milliseconds.							

Data are in milliseconds.

filtering and summing 3 blocks of 1,000 sweeps. The upper curve corresponds to EABRs elicited from the left ear, which had experienced a shorter ΔtD . The lower curve corresponds to recordings from the right ear.

Waves III and V could easily be identified for each pair of electrodes. Measurements made 6 months after implantation revealed similar patterns for the 3 pairs.

The Table contains EABR wave III and V latencies obtained in case 1, with mean values and standard deviations. The interaural shift in wave latencies was computed for each bilateral pair of electrodes. Comparison between right- and left-side electrodes showed no noticeable difference in wave III latency, but a remarkable difference in wave V latency. Whatever the pair of tested electrodes and the test time, wave V was delayed in the ear with the greater ΔtD .

Case 2. A 35-year-old man had been operated on for a left cholesteatoma at the age of 6 years. His left-side hearing was moderately impaired at that time. He became profoundly deaf on the right side when he was 10 years old, as a result of meningitis (Fig 5). When he was 15 years old, a decision was made to correct his moderate left-sided hearing loss with a conventional hearing aid. In 1984, he suddenly lost his residual left hearing function. At the time of surgery, he had been profoundly deaf on the right side for 25 years, and on the left side for 15 years. The electrophysiological measurements were performed at 6 and 9 months after surgery.

At both testing times, reproducible evoked potentials could be elicited in pairs 8-9 and 12-13, but not in pair 2-3. Figure 6 shows EABRs obtained in the

apical and midrange pairs at the postoperative interval of 9 months, the upper curve corresponding to the even electrode (left ear) and the lower to the odd one (right ear). There was no abnormality in EABR wave shape when we tested the even electrodes. The results were quite different for the odd ones, located on the side with the longer ΔtD . Here, wave V could also be identified. However, there was a lack of reproducibility of wave III when we tested electrode 13 at 6 months after surgery and when we tested electrode 9 at 6 and 9 months.

The wave latencies are presented in the Table, with the corresponding interaural shifts in wave V latency. The wave V latency was prolonged on all tests in the ear in which the ΔtD was greater.

DISCUSSION

This report is, to our knowledge, the first to investigate electrophysiological outcomes in patients with bilateral implants controlled by a single processor. It studies the characteristics of EABRs in 2 patients in which ΔtD differed from one ear to the other. For each patient, the EABR characteristics varied between the two ears, depending on the ΔtD . Good-quality EABR recordings could be obtained on the side with the shorter ΔtD . On this side, the EABR wave shapes and latencies were similar to those usually obtained in monaural cochlear implant patients, whether with the Digisonic system^{7,8} or with other cochlear implants.⁹⁻¹¹ Note that the wave V latency was lengthened on the side with the greater ΔtD .

The ΔtD in postlinguistically deaf adult cochlear implant patients is thought to be a major predictive factor of cochlear implantation outcome.¹²⁻¹⁵ However, no direct relationship has yet been demonstrated

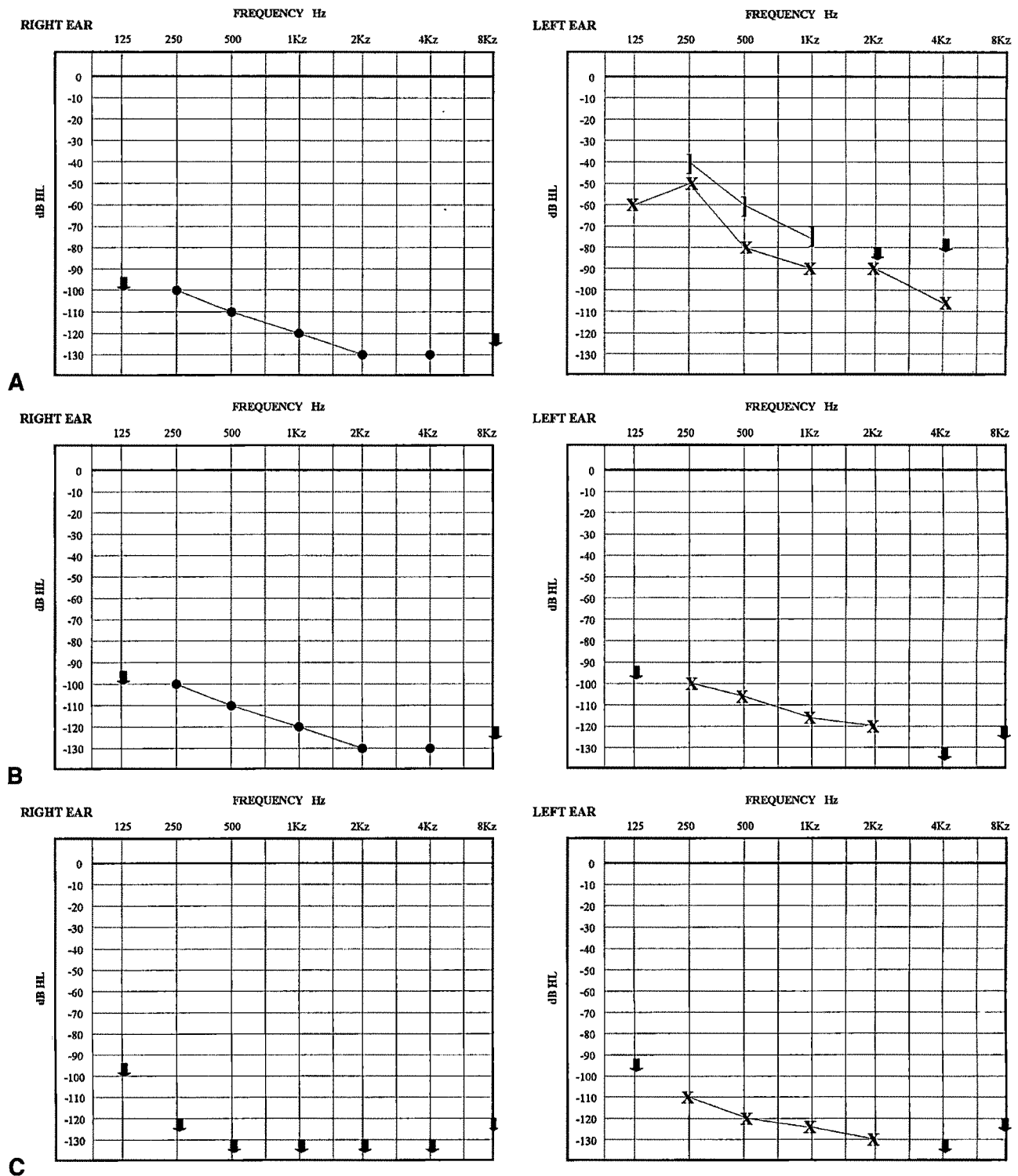


Fig 5. (Subject 2) Pure tone audiograms performed in A) 1983, B) 1984, and C) 1999.

in humans between ΔtD and EABR characteristics. What has been demonstrated is the influence of the ΔtD on neural survival in mammalian brain stem auditory nuclei.^{16,17} Several results in the literature support the notion that neural survival might be reflected in the growth of EABRs.¹⁸⁻²⁰ Histologic examinations of human temporal bones have revealed that

spiral ganglion cell counts are lower in old than in young deaf subjects and are inversely related to ΔtD .²¹ Furthermore, EABR thresholds are elevated in cats with extensive acoustic nerve damage.²² Studies conducted in animals have also shown that long-term profound deafness can decrease the inferior colliculus response to electrical stimulation of the cochlea.²³

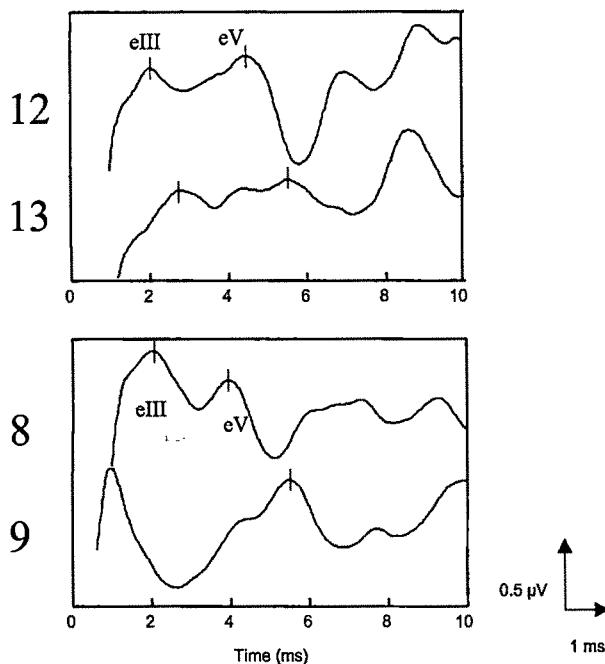


Fig 6. (Subject 2) EABRs obtained at postoperative interval of 9 months for apical (12-13) and midrange (8-9) electrodes. Upper curves correspond to recordings from ear with shorter deafness duration, ie, left ear.

Taken together, these findings suggest that EABR alterations reflect the effect of the ΔtD on neural response properties in the peripheral and intermediate auditory system.

To date, changes in auditory pathway activation induced by prolonged deafness, as assessed by EABRs, have only been supported by interindividual differences in trace quality. Bilateral cochlear implantation provides an interesting model for studying the effects of the ΔtD on brain stem auditory pathway activation within the same subject.

In the cases reported here, there was an interaural

shift in wave V latency. In 1 case, wave III was not reproducible on the side with the greater ΔtD . One interpretation would be that damage to auditory structures is likely to worsen with time, and may therefore differ from one side to the other in the same subject. The absence of a noticeable shift in wave III latency underlines the fact that wave V more accurately reflects any change in brain stem auditory pathway activation.

The observed results could also be linked to differences in the cause of deafness between the two ears. Spiral ganglion cell populations in ears with sudden deafness are larger than those in ears deafened by infectious labyrinthitis or genetic or congenital causes.^{21,24} This difference could account for some of the interaural difference in EABR characteristics observed in case 2. However, in case 1, the cause of deafness was the same for both ears, and therefore cannot account for the interaural EABR differences.

In these 2 cases, suppose that the differences in EABR are mainly due to asymmetric ΔtD . Then, does a poor EABR on one ear result from the total ΔtD in this ear, or does it result from the length of time for which it was the patient's only profoundly deaf ear? One may hypothesize that neural transmission on a deaf side deteriorates over time, not only because of a lack of activation, but also because of compensation by the other ear. This last hypothesis would need to be further investigated in large studies of bilateral cochlear implant patients with both 1) a wide range in the time interval, ΔtD , from the onset of profound deafness and 2) asymmetry in the duration of profound deafness for the left and right ears. Should such studies accompany the growing use of bilateral cochlear implantation, they would help in selecting the most appropriate candidates for this kind of procedure.

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DNA PLOIDY STATUS AS A PROGNOSTIC MARKER AND PREDICTOR OF LYMPH NODE METASTASIS IN LARYNGEAL CARCINOMA

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In patients with laryngeal carcinoma, nodal metastasis, recurrence after radiotherapy, and prognosis are important factors in clinical decision-making. Parameters such as tumor stage are considered insufficient for predicting these important items. The DNA ploidy status of the tumor may be a useful additional marker. The DNA ploidy status of 38 laryngeal cancers was determined by flow cytometry. Correlations were studied with TNM stage, differentiation, survival rate, relapse risk, recurrence after radiotherapy, and nodal metastasis. A positive correlation of DNA ploidy status with the development of lymph node metastases was found for diploid and peridiploid versus aneuploid tumors (DNA index, <1.4 versus ≥ 1.4 ; $p = .007$). No correlation was found between ploidy status and recurrence after radiotherapy. The overall survival rate ($p = .01$), but not the disease-specific survival rate or the relapse risk, showed a correlation with the ploidy status. The DNA ploidy status may be a useful marker for metastatic behavior in head and neck squamous cell carcinoma and may therefore be helpful in decision-making concerning elective treatment of the neck.

KEY WORDS — flow cytometry, larynx, metastasis, ploidy status, squamous cell carcinoma.

INTRODUCTION

Metastatic behavior and recurrence after radiotherapy are important issues in making decisions on the treatment of patients with head and neck squamous cell carcinoma (HNSCC). But patients and their tumors, even of similar stage, are heterogeneous. Tumors may show a wide range of characteristics concerning growth, metastatic behavior, response to different treatments, and prognosis. If patients could be better selected for the appropriate treatments, the outcome might be improved. In patients with HNSCC, choices of treatment are currently mainly based on TNM stage. But TNM staging cannot predict the behavior of individual tumors, and there are no tools to predict this behavior more reliably. So, a need is felt for additional parameters.

The potential clinical relevance of finding markers for lymph node metastasis is considerable. Regional metastasis is an important factor in the treatment and prognosis of patients with HNSCC, but the accuracy of current diagnostic methods to detect these metastases is insufficient. For the detection of metastases by palpation or imaging techniques, a minimum size of these metastases is required. Therefore, small and only microscopically detectable metastatic deposits will not be recognized, and uncertainty about the true

lymph node status of the neck will remain. Even ultrasound-guided fine-needle aspiration biopsy, the most accurate technique to detect lymph node metastases to date, identifies clinically occult metastases with a sensitivity of no more than 48% to 76%.^{1,2} Because of the high false-negative rates, most head and neck oncologists will electively treat the neck of patients with tumors with a presumed high propensity to metastasize. Consequently, many patients will receive an unnecessary treatment for their neck, with concomitant risk of mortality and morbidity.^{3,4} If the probability of regional metastases can be reduced, the number of elective neck treatments can also be diminished.⁵

Some tumors respond favorably to radiotherapy, and others do not. These latter cases should perhaps be treated with initial surgery, eg, laser surgery, or should be monitored more carefully after irradiation. Although, in the literature, factors have been described that may supply information, there are no reliable criteria available to predict recurrence after radiotherapy to date.

Chromosomal changes and histologic features of tumors can provide valuable information concerning the probability of nodal metastasis^{6,7} or recurrence after radiotherapy in HNSCC.⁸⁻¹¹ However, the re-

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TABLE 1. POPULATION CHARACTERISTICS OF 38 PATIENTS WITH HNSCC

Level	
Glottic	27
Supraglottic	8
Both or transglottic	3
Primary treatment	
Surgery and radiotherapy	12
Radiotherapy alone	26
T stage	
1	13
2	13
3	10
4	2
N status	
Positive	9
Negative	29
Sex	
Male	33
Female	5
Age (y)	
Mean	60.2
Range	12-81

HNSCC — head and neck squamous cell carcinoma.

sults of different studies are often conflicting, and no marker in particular has yet been established for clinical use. The above-mentioned and other applications of biomarkers, like cancer development risk and relapse risk assessment, were reviewed by Oh and Mao.¹² Despite the fact that the molecular mechanisms of carcinogenesis are not yet fully understood, biomarkers may help to select patients for certain treatments, eg, elective neck treatment.^{6,7} If, in addition to imaging techniques, biomarkers can further predict the probability of nodal metastasis, it may be decided not to treat the neck electively.

The ploidy status of tumors reflects the DNA content of the tumor cells. Aneuploidy is the result of failing mitotic checkpoints.¹³ According to the literature, the ploidy status of tumors may be a useful biomarker and may prove helpful in the above-mentioned clinical decision-making process in patients with HNSCC.¹⁴⁻²³

We studied the correlation of ploidy status with the development of regional metastasis, recurrence rates after radiotherapy, and survival rates in 38 patients with laryngeal carcinoma with a follow-up of at least 10 years.

MATERIALS AND METHODS

Paraffin-embedded pretreatment biopsy material of 38 patients who presented with laryngeal carcinoma in the period 1973 to 1982 at Leiden University Medical Center was used. The population characteris-

tics are shown in Table 1. The patients were all treated with either initial radiotherapy or surgery (total laryngectomy) and radiotherapy. Follow-up was at least 10 years. The patients in the lymph node-positive group had histologically proven lymph node metastases either initially or in the follow-up (regional recurrence). The patients in the lymph node-negative group had no detectable metastases, either initially or in the follow-up period.

Flow Cytometry. Cell preparation and staining procedures have been described elsewhere.²⁴ The pepsin-digestion technique (0.5% pepsin in saline solution, pH 1.5 for 30 minutes) was used to release nuclei from 40- to 45- μ m-thick sections of paraffin-embedded tissue specimens, and they were stained with propidium iodide. The DNA content was determined with a FACScan flow cytometer (Becton and Dickinson, Mountain View, California). On average, about 10,000 cells were measured in each sample. Tumors with a DNA index of more than 1.00 were classified as aneuploid. To discriminate between diploid or peridiploid tumors and tumors with more severe aneuploidy that probably arose via polyploidization, a cut-off point of 1.4 was also made. The cutoff of 1.4 was used to discriminate between near-diploid and hypotetraploid tumors and represents the minimum in the bimodal DNA index frequency that is a well-documented finding in breast cancer. This distribution is suggested to be a reflection of different mechanisms of ploidy evolution.²⁵

Statistical Analysis. We investigated the correlation between DNA ploidy status, T stage, and nodal status with overall and disease-specific survival rates and relapse risk using Cox regression analysis. Actuarial survival plots were calculated according to the Kaplan-Meier method. The date of treatment was taken as the starting point for this analysis. The overall survival rate was calculated by counting as dead all patients who died irrespective of the cause, and the disease-specific survival rate was calculated by counting as dead all patients who died of their laryngeal cancer. The relationship between DNA ploidy status and stage, differentiation, recurrence after radiotherapy, and lymph node metastases was tested with the χ^2 test. Correlations or differences with a p value of less than .05 were considered statistically significant.

RESULTS

The DNA index ranged from 1.0 to 2.78. The DNA index frequency distribution is shown in Fig 1. The coefficient of variation ($\pm$ SD) was 7.1 ± 3.03 . Of the 38 investigated cases, 23 (61%) were aneuploid if only tumors with a DNA index of 1.00 are considered as diploid. If the diploid and peridiploid tumors are

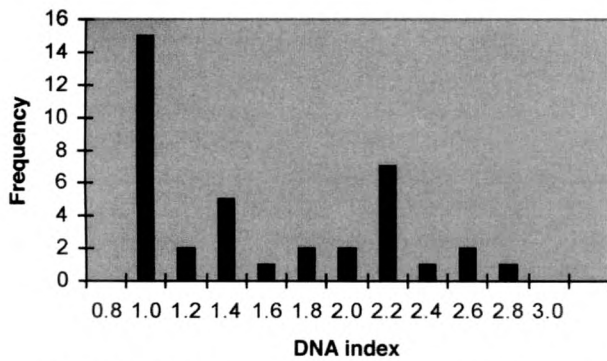


Fig 1. DNA index frequency distribution of 38 cases of laryngeal carcinoma.

taken together, 18 of the cases (47%) were aneuploid.

With 1.4 as a cutoff point, a significant correlation of ploidy status with lymph node metastases was found ($p = .007$). In the diploid-peridiploid group, 1 of 20 patients (5%) had lymph node metastases, whereas in the aneuploid group, 10 of 18 of the patients (56%) had metastases. With the cutoff point at 1.0, the correlation was nearly significant ($p = .06$; Table 2).

In the 26 patients treated with primary radiotherapy, no correlation was found between ploidy status and recurrence after radiotherapy with either of the cutoff points ($p = .69$ for 1.0 and $p = 1.00$ for 1.4).

The DNA ploidy status of the tumors was not related to the T stage ($p = .67$ for 1.0 and $p = .51$ for 1.4) or overall UICC TNM stage grouping ($p = .36$

for 1.0 and $p = .23$ for 1.4). When diploid and peridiploid tumors were set against aneuploid tumors (cutoff point of 1.4), a correlation was found with the differentiation of the tumor ($p = .03$).

Table 3 summarizes the influence of aneuploidy (diploid or peridiploid versus aneuploid), T stage, and nodal status on survival rate, disease-specific survival rate, and relapse risk. As is shown by the Cox regression multivariate analysis, aneuploidy has a significant prognostic influence on overall survival rate (hazard ratio, 3.47; $p = .01$), independent of T stage, nodal status, differentiation, or age. In the aneuploid group, survival rate was significantly worse than in the diploid-peridiploid group. The results with diploid versus peridiploid and aneuploid tumors showed a similar difference in survival rate (hazard ratio, 2.89; $p = .03$). T stage (T1 versus T2-T4; $p = .20$) did not correlate with overall survival rate, and neither did nodal status ($p = .79$). For disease-specific survival rate and relapse risk, none of the investigated variables was significant. For disease-specific survival rate, T stage came nearest to significance.

Figure 2 gives the Kaplan-Meier plot for the influence of aneuploidy (diploid or peridiploid versus aneuploid) on overall and disease-specific survival rates.

Additional analysis of our data showed that in the diploid group, of the 6 patients who died, 2 died of intercurrent disease. In the aneuploid group, 12 of the 17 deaths were of intercurrent disease. Thus, in

TABLE 2. RELATIONSHIP BETWEEN PLOIDY STATUS AND DEVELOPMENT OF LYMPH NODE METASTASES, RECURRENCE AFTER RADIOTHERAPY, T STAGE, AND OVERALL STAGE GROUPING ACCORDING TO UICC CATEGORIES FOR TWO CUTOFF POINTS OF DNA INDEX, 1.0 AND 1.4

	Cutoff at 1.0			Cutoff at 1.4		
	Diploid	Aneuploid	<i>p</i>	Diploid	Aneuploid	<i>p</i>
Differentiation			.16			.03
Poor	0	2		0	2	
Moderate	7	15		9	13	
Well	8	6		11	3	
N-	14	15	.06	19	10	.007
N+	1	8		1	8	
No recurrence	6	9	.69	9	7	1.00
Recurrence	6	5		7	4	
T1	6	7	.67	9	4	.51
T2	5	8		6	7	
T3	4	6		4	6	
T4	0	2		1	1	
I	6	6	.36	9	3	.23
II	5	7		6	6	
III	4	6		4	6	
IV	0	4		1	3	

UICC — Union Internationale Contre le Cancer, N- — no lymph node metastasis; N+ — lymph node metastasis.

UICC staging categories: stage I is T1N0M0; stage II is T2N0M0; stage III is T3N0M0, T1N1M0, T2N1M0, or T3N1M0; and stage IV is T4N0-1M0, T+N2-3M0, or T+N+M1.

TABLE 3. INFLUENCE OF ANEUPLOIDY (DIPLOID OR PERIDIPLOID VERSUS ANEUPLOID), T STAGE, AND NODAL STATUS ON SURVIVAL RATE, DISEASE-SPECIFIC SURVIVAL RATE, AND RELAPSE RISK BY COX REGRESSION ANALYSIS

		N	Overall Survival (23 Events)	Disease-Specific Survival (9 Events)	Relapse Risk (14 Events)
Aneuploidy	<1.4	20	1.00	1.00	1.00
	≥1.4	18	3.47 (1.35-8.92) p = .01	1.55 (0.35-6.86) p = .57	0.93 (0.28-3.10) p = .90
T	1	13	1.00	1.00	1.00
	2-4	25	1.87 (0.71-4.90) p = .20	6.05 (0.74-49.5) p = .09	1.14 (0.37-3.52) p = .82
N	pN-	29	1.00	1.00	1.00
	pN+	9	0.87 (0.31-2.43) p = .79	1.60 (0.32-7.92) p = .56	1.80 (0.48-6.84) p = .38

Data are hazard ratios, with 95% confidence intervals in parentheses.

the aneuploid group, more patients died of intercurrent disease. Age correlated with ploidy status nearly significantly ($p = .08$, t -test). The average age in the diploid group was 55.7 years, versus 63.1 years in the aneuploid group. For diploid and peridiploid versus aneuploid tumors, this difference was less pronounced ($p = .18$, t -test). Thus, there is a trend of more aneuploid tumors at a higher age. However, in Cox regression multivariate analysis, ploidy status had prognostic significance independent of age.

DISCUSSION

In this study, the ploidy status of laryngeal cancers

correlated with the development of nodal metastasis, but not with recurrence after radiotherapy. Moreover, it showed a correlation with overall survival rate, but not with disease-specific survival rate or relapse risk.

In recent reports on DNA ploidy status in HNSCC, correlations have been studied with clinical parameters such as stage and metastasis. In studies of HNSCC of different sites, a correlation with stage or nodal metastasis was found in some studies,^{16,25} but in other studies, this correlation could not be established.^{26,27} A correlation with lymph node metastasis in carcinomas of the oral cavity was found by several

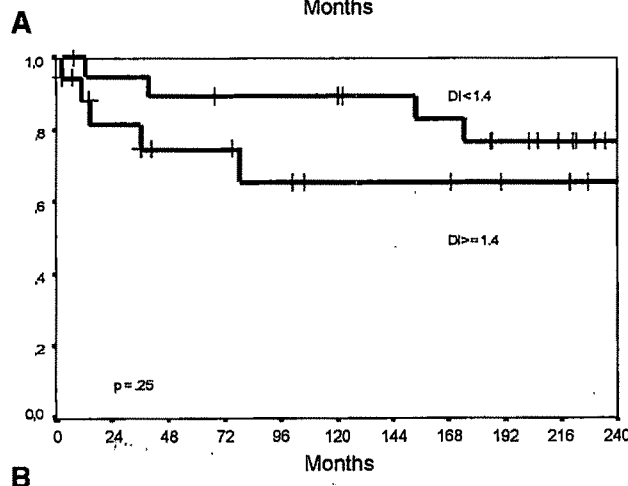
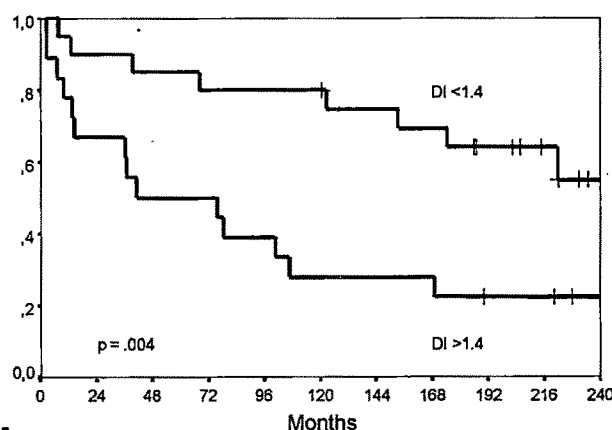
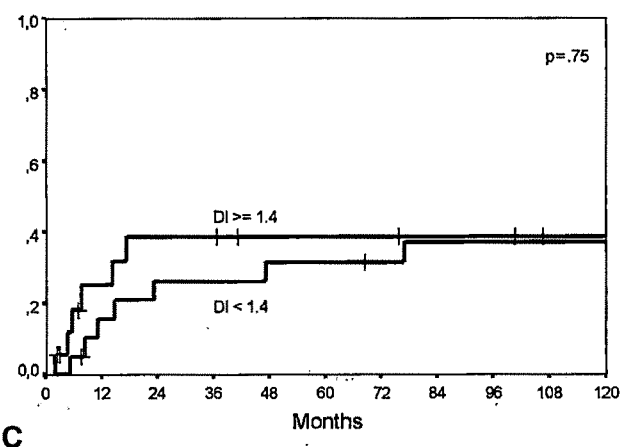


Fig 2. Kaplan-Meier plots of A) overall survival rate, B) disease-specific survival rate, and C) relapse risk for ploidy status with 1.4 as cutoff point (log rank test). DI — DNA index.



authors,^{15,17,19,20,22} although others found only a weak correlation.⁷ As did we, Wolf et al¹⁸ found a higher rate of lymph node metastases in aneuploid laryngeal carcinomas. So, both in our study and in the literature, DNA ploidy appears to correlate with nodal metastasis.

DNA ploidy may be a useful biomarker for the development of lymph node metastasis, as a single marker or in combination with other relevant parameters and diagnostic techniques. Such a combination of relevant factors may achieve even higher reliability in detecting or predicting lymph node metastases.^{6,7} In cases with no clinically or radiologically detectable nodal metastases, a further lowered probability of micrometastases based on markers like DNA ploidy could support a decision not to perform elective neck treatment.⁵

A correlation of DNA aneuploidy with a higher risk of local recurrence after radiotherapy has been described in glottic carcinoma.²¹ We did not find a correlation between ploidy status and recurrence after irradiation. This result is consistent with the results of Pekkola-Heino et al,²⁸ who did not find an association of ploidy status with radiosensitivity in HNSCC cell lines. Other markers, in particular p53 and bcl-2, have also been studied in this respect, but with conflicting results.^{8-11,29-31} For example, in some studies, nuclear accumulation of p53 did not appear to be a significant predictor for control with radiotherapy in laryngeal carcinomas^{10,30} or HNSCC in general.³¹ Other studies did find a correlation between nuclear accumulation or mutation of p53 and recurrence after radiotherapy in HNSCC patients,^{8,11,32} whereas mutations of p53 in oral cavity cell lines appear to render the tumor cells more radiosensitive.¹⁰ Expression of bcl-2 in HNSCC was demonstrated to give better local control after accelerated radiotherapy.⁹ In conclusion, the results of studies of markers for predicting recurrence after radiotherapy are conflicting, and it is therefore unlikely that they will play a role in clinical decision-making in the near future.

ical decision-making in the near future.

Numerous markers have been studied for their prognostic significance. For DNA ploidy status, the results are again conflicting. Some authors did not find a statistically significant correlation with prognosis in HNSCC⁹ or squamous cell carcinoma of the oral tongue,³³ supraglottic larynx,³⁴ or pyriform sinus.³⁵ Others did find a correlation with both relapse-free and/or overall survival rate in HNSCC.^{14,23,36} In laryngeal carcinomas, a correlation with relapse-free survival rate has also been described.¹⁸

In our material, we found a correlation with overall survival rate, but not to disease-specific survival rate. This outcome may seem difficult to explain. However, the distinction between the two types of survival rate is not always clear in practice. Often, factors related to the treatment of the disease, eg, extensive surgery, may lead to death. Also, if the condition of the patient deteriorates because of progression of the disease, the probability of death by intercurrent diseases may increase as well. We observed a trend toward a high frequency of aneuploid tumors at a higher age. The correlation between ploidy status and overall survival rate therefore may be influenced by the fact that aneuploidy is associated with higher age, resulting in a higher chance of dying of intercurrent diseases. So, it seems that the ploidy status of the tumor does not have a direct prognostic significance in our study.

CONCLUSIONS

The DNA ploidy status appears to be correlated with lymph node metastasis and overall survival rate, but not with disease-specific survival rate or relapse risk, in laryngeal carcinomas. It may be useful in clinical decision-making concerning the treatment of the neck. Although, even as a single marker, it shows a significant correlation with lymph node metastases, the reliability of predicting lymph node metastases may be improved by using it in addition to other relevant parameters and imaging techniques.

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INCIDENCE OF COCHLEAR INVOLVEMENT IN HYPERBILIRUBINEMIC DEAFNESS

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Neonatal hyperbilirubinemia remains an important cause of childhood deafness, especially in developing countries. After neonatal hyperbilirubinemia, the auditory neural pathways, cochlea, or both may be affected. In this study, we aimed to determine the incidence of cochlear impairment and the appropriate means of hearing screening in hyperbilirubinemic neonates. A retrospective review of 1,032 pediatric patients with hearing loss revealed 67 cases (6.5%) of severe hyperbilirubinemia in the neonatal period. Thirty of these patients had neonatal hyperbilirubinemia as the single identifiable risk factor for hearing loss. In 26 of 30 cases (87%), otoacoustic emissions (OAEs) were absent, whereas in the remaining 4 cases (13%), robust emissions were detected despite an absent auditory brain stem response (ABR). Auditory screening of newborns with jaundice by OAEs possesses a significant risk of undiagnosed deafness. On the other hand, if the ABR is used as the single means of screening, auditory neuropathic conditions will probably be underlooked. Therefore, we recommend dual screening of hearing by ABR and OAEs in hyperbilirubinemic newborns.

KEY WORDS — auditory brain stem response, auditory neuropathy, deafness, neonatal hyperbilirubinemia, otoacoustic emissions.

INTRODUCTION

Hearing loss as a complication of bilirubin encephalopathy first received attention in the mid-1900s,¹ and since then, neonatal hyperbilirubinemia has been accepted as one of the main risk factors in childhood deafness. Unconjugated bilirubin readily crosses the blood-brain barrier in the neonatal period and can precipitate in different structures in the central nervous system and probably in the inner ear, which is on the same side of the barrier as the brain.

The nature and the location of the hearing loss caused by neonatal hyperbilirubinemia are still controversial, and researchers have pointed to different levels of the auditory pathway as the source of impairment. Morphological observations have suggested that the auditory impairment could be situated in neural pathways, in the cochlea, or in both. Some histopathologic studies of the ears of kernicteric neonates demonstrated the inner ears to be normal, whereas others showed severe damage in the cochlea.^{2,3} Anatomic studies of the central nervous system of kernicteric patients showed damage in the inferior colliculus and dorsal and ventral cochlear nuclei.^{2,4,5}

With the development of auditory brain stem response (ABR) testing and, later, otoacoustic emission (OAE) testing, researchers have focused on identifying the lesion site functionally. Chisin et al⁶ were the first researchers who attempted to localize the

auditory system lesion electrophysiologically. In their study, they found that a neural response from the auditory nerve was absent in 11 of 13 patients and was present only in response to high-intensity stimuli in the other 2 patients. In the majority of cases, a cochlear microphonic potential representing hair cell activity was present. Later, Perlman et al⁷ found aberrations of the ABR and prolonged brain stem transmission time in infants with jaundice who had serum bilirubin levels between 15 and 25 mg/dL. The discovery of practical methods for OAE measurement has made direct assessment of outer hair cell activity possible. This audiometric tool led to the identification of a distinctive hearing disorder in hyperbilirubinemic infants in the last decade of the 20th century.⁸⁻¹⁰ In 3 studies on OAE measurements, a total of 6 cases of hearing loss due to bilirubin toxicity were reported, and in all of these cases, OAEs were present despite the absence of an ABR.⁸⁻¹⁰ However, Sheykholeslami and Kaga¹¹ reported the absence of OAEs in 3 cases of hyperbilirubinemic deafness.

All of these research efforts make clear that after neonatal hyperbilirubinemia, the auditory neural pathways or the cochlea or both may be affected. However, the incidence of cochlear sparing in neonatal hyperbilirubinemia is still vague in the literature. The number of cases investigated with OAEs is currently limited and is far from clarifying the incidence of pure retrocochlear involvement. The objective of this study was to determine the incidence of cochlear im-

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pairment in a relatively large group of patients with the single risk factor of neonatal hyperbilirubinemia.

MATERIAL AND METHODS

A retrospective review of 1,032 pediatric patients with hearing loss followed at the Istanbul School of Medicine Center for Deaf Children revealed that 67 patients (6.5%) had severe hyperbilirubinemia in the neonatal period. Thirty of these 67 patients had neonatal hyperbilirubinemia as the single risk factor for the sensorineural hearing loss. All of the subjects fulfilled the following criteria: 1) history of severe hyperbilirubinemia in the neonatal period; 2) uncomplicated pregnancy and perinatal period; 3) normal fetal growth and absence of congenital abnormalities; and 4) absence of parental consanguinity or family history of deafness.

The patients were enrolled for this study by telephone call. Seventeen of them were girls, and 13 were boys. The mean ($\pm$ SD) age was 32 ± 9.3 months. The mean gestational age was 38.2 ± 1.1 weeks, and the mean birth weight was $3,044 \pm 24.9$ g. The causes of neonatal hyperbilirubinemia were Rh incompatibility in 11, unknown in 11, and ABO blood group incompatibility in 8. The mean peak serum bilirubin level was 24.4 ± 8.8 mg/dL in the neonatal period.

Otoscopic examination and tympanometry were performed in all subjects. Auditory brain stem responses were investigated with click stimuli in alternating polarity. Active electrodes were attached to the ipsilateral mastoid region, and the reference electrode was attached to the vertex. Both ears were tested independently by earphones in order to estimate hearing thresholds. A Celesta 503 Cochlear Emissions Analyzer (Madsen Electronics, Taastrup, Denmark) was used to test distortion product OAEs (DPOAEs). The f1- and f2-stimulating frequencies were presented at 70 dB sound pressure level (SPL) simultaneously. The f2/f1 ratio was kept at 1.22, and the 2f1 - f2 DPOAEs were recorded at geometric center frequencies 0.75, 1, 1.5, 2, 3, 4, 6, and 8 kHz and displayed as a DP-gram. The DPOAEs were considered to be present if they were at least 6 dB greater than the noise floor. Both ABR and DPOAE recordings were performed in a silent room with the patients asleep.

RESULTS

In 30 cases of sensorineural hearing loss, the impairment was bilateral in 28 and unilateral in the remaining 2. The hearing loss was found to be profound (>90 dB HL) in 20 cases, severe (70 to 90 dB HL) in 6 cases, and mild to moderate (<60 dB) in 4 cases. No discernible waveform was seen at ABR testing of the 20 subjects with profound hearing loss.

In the 6 patients with severe hearing loss, the absolute latencies of wave I and wave V were increased and I-V interwave latencies were preserved, while in the 4 patients with mild to moderate hearing loss, the absolute and interwave latencies were normal on ABR testing.

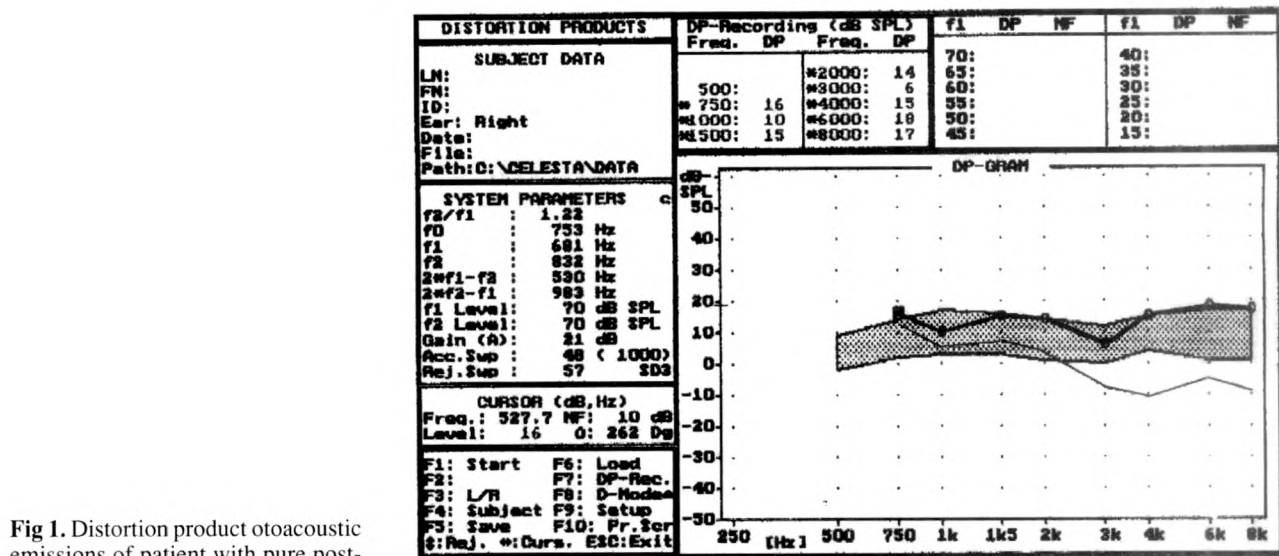
Before OAE measurement, the otoscopic and tympanometric findings were normal in all cases. Distortion product OAEs were absent at all test frequencies (750 Hz to 8 kHz) in 26 of the 30 cases (87%). Among these 26 cases, 2 patients had unilateral hearing loss, and DPOAEs were absent on the deaf side. Robust emissions were detected in 3 of the remaining 4 cases, whereas in 1 case, DPOAEs were absent at the higher frequencies (3 to 8 kHz). In these 4 cases with normal DPOAEs (Fig 1), no discernible waveform was detected to a maximum click stimulus on the ABR test (Fig 2).

DISCUSSION

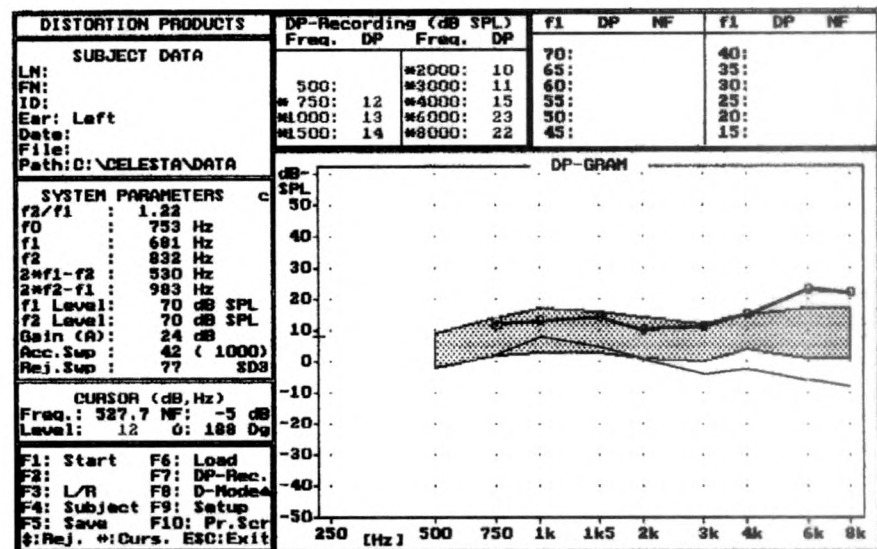
The incidence of deafness due to neonatal hyperbilirubinemia has been decreased by improved perinatal care and by the use of treatments such as anti-D antibody, phototherapy, and exchange transfusions. However, neonatal hyperbilirubinemia still remains an important cause of childhood deafness in developing countries. According to our series, in 6.5% of all children with deafness, severe neonatal hyperbilirubinemia can be found as a contributing factor.

Unconjugated bilirubin with elevated serum levels readily crosses the blood-brain barrier and accumulates in neural structures and in the inner ear, as well. The location of hyperbilirubinemic deafness is still controversial; the inner ear, auditory nerve, and brain stem may all be involved sites. Several human and animal studies have appeared in the literature to support each possible site of involvement. Anatomic studies in Gunn rats with deficient liver enzymes showed the cochlear nucleus and inferior colliculus as the sites of involvement.^{4,12} Some human studies demonstrated destruction of ventral and dorsal cochlear nuclei, while others found inner ear destruction without central nervous system pathology.^{3,13} Electrophysiological investigations of the auditory neural pathways strongly support a neural cause in deafness due to neonatal hyperbilirubinemia. Chisin et al⁶ found absence of a neural response from the auditory nerve in 11 of 13 patients, and in the majority of cases, cochlear microphonic potentials were present. Subsequently, Perlman et al⁷ reported prolonged brain stem transmission times in infants with jaundice.

Otoacoustic emissions are believed to originate from the active mechanical forces generated by the



A



B

outer hair cells, and in their presence, the cochlea is presumed to be healthy.^{14,15} In 1993, Lafreniere et al¹⁰ first reported the presence of OAEs in newborns with severe hyperbilirubinemia. In that study, no other waveforms besides a reproducible wave I was obtained on ABR with an 85-dB stimulus. Likewise, Rhee et al⁸ demonstrated the presence of OAEs in 4 cases in the absence of an ABR, and Simmons and Beauchaine⁹ reported the same finding in 1 case. However, in 3 of these 6 reported cases, neonatal septicemia and prolonged respiratory distress were additional risk factors that may have contributed to the hearing disorder. So far, the absence of OAEs has only been reported by Sheykhosslami and Kaga¹¹ in 3 cases of hyperbilirubinemic deafness. These latter reports⁸⁻¹¹ are single-case reports or studies made on a limited number of cases, and their results are far from showing the incidence of cochlear involvement.

Our study diverges from the previous ones in 2 ways: 1) hyperbilirubinemia was the only risk factor that could be determined, and the results were not contaminated by other causes of deafness; and 2) the study was made in a relatively large group of subjects, thereby providing an opportunity to determine the incidence of cochlear sparing. In our study, only in 4 of the 30 patients (13%) were the DPOAEs within the normal range, despite no discernible ABR waveforms. In other words, in hyperbilirubinemic deafness, the disease was purely postsynaptic in 13% of cases, and in the remaining cases, definite cochlear involvement was found. The possible explanation of the complete absence of ABR waveforms in these cases could be the lack of an ability of the auditory pathway neurons to respond synchronously to an input signal.¹⁶ In 26 of 30 cases (83%), obvious cochlear impairment was documented by the absence of DPOAEs. In 10 patients with ABR responses to

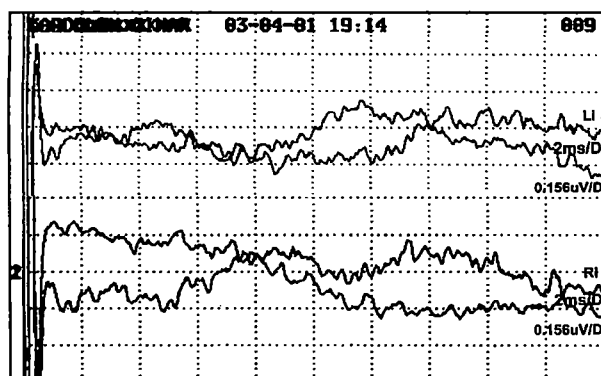


Fig 2. Auditory brain stem response shows no discernible waveform to click stimulus of 100 dB. Li — left ipsilateral; Ri — right ipsilateral.

higher stimulus levels, interwave latencies were preserved, indicating a normal brain stem conduction time. In the remainder of the cases, plausible bilirubin toxicity to the auditory brain stem pathway could not be ruled out because of the masking effect of peripheral disease.

Three years after the first case of Lafreniere et al,¹⁰ Starr et al¹⁶ reported 10 cases of mild to severe pure tone hearing loss, poor speech discrimination, absent ABRs, and normal OAEs and named this disorder auditory neuropathy (AN). Charcot-Marie-Tooth disease, Friedreich's ataxia, and Guillain-Barré syndrome are other known causes of AN.^{9,16} Mild to moderate hearing losses are found on pure tone audiometry in most AN patients, but their word recognition abilities are disproportionately poorer than their audiometric thresholds.¹⁶ Adult AN patients usually state that they can hear but cannot understand. There are even some patients who can hear the click stimuli during the ABR testing. The cause of this bizarre situation is probably altered neural synchronicity and impaired temporal encoding of speech.^{9,16} Absence of contralateral acoustic reflexes and contralateral suppression of OAEs are the other reported findings in AN cases.^{17,18} The audiometric findings show wide variety and the patient population is heterogeneous, but the single common finding is the presence of nor-

mal OAEs demonstrating that the lesion is definitely post-outer hair cell despite paradoxical absences of the ABR.¹⁶ Although word recognition tests and pure tone audiometry could not be done in our 4 patients because of their ages, the presence of OAEs with an abnormal ABR may suggest the diagnosis of AN.

In hyperbilirubinemic neonates, the determination of the incidence of pure post-outer hair cell disease — in other words, AN — is important in 2 main issues: in determining the best means of hearing screening, and in choosing the rehabilitation method for deafness. Otoacoustic emission testing has gained worldwide acceptance for newborn auditory screening because of its advantages such as reliability, rapidity, and low cost.^{19,20} However, according to our study, if OAE testing is chosen as the screening method in neonatal hyperbilirubinemia, 13% of cases will be undiagnosed. Therefore, its low sensitivity in neonatal hyperbilirubinemia appears to be a major drawback. On the other hand, auditory screening of these subjects with ABR testing will diagnose hearing loss with high accuracy, but it will probably miss pure postsynaptic hearing losses such as AN, the remediation of which still remains controversial. Amplification is disregarded by some authors because of its potential hazards for the outer hair cells and the lack of auditory nerve synchronicity in this patient group.¹⁶ Similarly, cochlear implantation in such cases is questionable because of unpredictable post-implantation performance.²¹

CONCLUSION

The incidence of cochlear sparing in hyperbilirubinemic deafness appeared to be 13% in this study. Therefore, auditory screening of newborns with jaundice with only OAE testing carries a significant risk of undiagnosed deafness. If ABR testing is used as the single method of screening, the same subjects will probably be misdiagnosed and inappropriate use of hearing aids will be recommended. For these reasons, we recommend dual screening with ABR and OAE testing in hyperbilirubinemic newborns.

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TRAVELING FELLOWSHIP

The Department of Otolaryngology-Head and Neck Surgery at the Hospital Europeen Georges Pompidou, University of Paris V, France, together with the association Progres 2000, is sponsoring a traveling fellowship starting May 1, 2003. A 2-month scholarship will provide extensive training in laryngology-head and neck surgery and oncology every year during the months of May and June. The successful candidate will be an active member in the department and participate in research. For further information, contact ollivier.laccourreye@hop.egp.ap-hop-paris.fr.

ROLE OF MEDIALIZATION IN THE IMPROVEMENT OF BREATH CONTROL IN UNILATERAL VOCAL FOLD PARALYSIS

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A prospective study was performed on 18 adults to analyze disturbances of voice and respiratory function in unilateral vocal fold paralysis and the role of medialization in the improvement of breath control. In addition to physical examination, an acoustic and aerodynamic analysis, as well as patient self-assessment, were performed both before and after medialization (14 fat injections, 4 thyroplasties) to evaluate voice and both phonatory breath control and respiratory function outcome. Preoperative and postoperative results, at short, middle, and long term, were compared to data from 14 control subjects and evaluated by statistical analysis. Preoperative acoustic and aerodynamic parameters were significantly disturbed in comparison to controls. Independently of the pulmonary status, medialization induced an improvement in most of the parameters at short and long terms, whereas a diminished effect was observed at middle term. Phonatory breath control, as evaluated by maximum phonation time, mean flow rate, and expired volume, was statistically improved, with the best results being obtained in women. From the questionnaire, it was clear that voice, as well as phonatory breath control and respiratory function, showed considerable improvement, although some difficulties persisted during effort. This study demonstrates that there is a disturbance of phonatory breath control and respiratory function in adult unilateral vocal fold paralysis. Improvement following medialization supports the concept that the respiratory dysfunction seen beforehand is most probably the result of air escape rather than laryngeal obstruction. Although medialization may provoke an extrathoracic obstruction, its feature of variability appears to improve breath control.

KEY WORDS — dysphonia, medialization, phonatory breath control, respiratory function, vocal fold paralysis.

INTRODUCTION

Unilateral recurrent nerve paralysis results in inappropriate vocal fold vibration leading to dysphonia, which is usually the major symptom of the paralysis. Although swallowing disorders are commonly reported in cases of 10th cranial nerve paralysis, in which both superior and inferior laryngeal nerves are impaired, incompetence of the laryngeal sphincter, in addition to pharyngeal dysfunction, may disturb airway protection in recurrent nerve paralysis.¹⁻⁶ Besides its airway protective function, the respiratory function of the larynx is important, since it regulates to some degree the intrapulmonary pressure to prevent collapse of the lung.⁷ Modulation of subglottic pressure regulates phonation, and complete closure is necessary before an explosive cough. Although dyspnea has been described following unilateral laryngeal paralysis in infants^{8,9} (due to airway obstruction by the narrowing of the glottic area), in adults, symptomatic disturbed airflow has been more commonly reported as breathiness, affecting the phonatory breath, as evaluated by the GRBAS (grade, roughness, breathiness, asthenia, strain) scale.¹⁰ Some authors have, however, studied in adults the consequences of laryngeal obstruction on the respiratory function, both before and after vocal fold medializa-

tion.¹¹⁻¹⁴ By means of ventilatory function tests, it has been mostly related to a decrease in the laryngeal aperture during inspiration, with an increased airway obstruction following medialization,¹²⁻¹⁴ except in Kashima's study.¹¹ It was also pointed out that the decrease in mean peak inspiratory flow after medialization, with no change in expiratory flow, indicates that there is no fixed airway obstruction.¹⁴ In addition, Leder and Sasaki¹⁵ have reported the effectiveness of thyroplasty in maintaining an adequate subglottic pressure that allows an easy use of airflow during speech. One may suggest that a variable degree of upper airway obstruction, provoked by medialization, is required to produce an improvement in breath control. This suggestion supports the concept that greater inspiratory efforts during unilateral vocal fold paralysis are developed to compensate for the airway escape from the glottic aperture and the decreased laryngeal resistance. This escape may compromise the air volumes required for phonation and respiration.

To demonstrate the effect of these breath control disturbances in unilateral vocal fold paralysis, we prospectively studied 18 patients both before and after vocal fold medialization. Phonatory breath control and respiratory function were evaluated by aro-

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dynamic measures and by self-assessment. Vocal outcome was also studied, by analysis of acoustic parameters and self-assessment by the patient. The present study demonstrates that medialization promotes regulation of both phonatory breath control and respiratory function, and thus greatly improves the patient's quality of life.

PATIENTS AND METHODS

Eighteen of 23 patients consecutively referred to our institution for the rehabilitation of unilateral laryngeal paralysis were included in this prospective study. Ten patients were male, 8 were female, and their mean age was 57.5 years (range, 24 to 80 years). Four of them had sedentary occupations. Previous voice therapy had been ineffective or insufficient in all of them. The mean duration of the paralysis was 13.8 months (range, 4 to 40 months) before medialization. There were 13 recurrent nerve paralyses and five 10th cranial nerve paralyses, which were idiopathic in 3 cases and due to surgery in the others (skull base, thyroid, or thoracic surgery). The right vocal fold was paralyzed in 8 patients, and the left was paralyzed in 10 patients. Medialization of the paralyzed vocal fold was achieved by autologous fat injection in 14 cases (6 women and 8 men) and by thyroplasty with a silicone implant (fashioned from a silicone block) in 4 patients (2 women and 2 men). (One thyroplasty procedure was performed after autologous fat injection that had not greatly improved the condition.)

In addition to a physical examination including a videofiberscopic examination, an acoustic and aerodynamic analysis and a patient self-assessment were performed before and after medialization. The fiberoptic examination and acoustic and aerodynamic parameters were recorded within 2 weeks before vocal fold medialization, and also at periodic intervals after the surgery: from 1 to 3 months (short term; ST), from 4 to 6 months (middle term; MT), and from 7 to 12 months (long term; LT) after the procedure. The patient self-assessment was evaluated by a questionnaire before surgery and from 8 to 10 months after surgery. In addition, an acoustic and aerodynamic evaluation was performed in a control population of 14 adults (8 men and 6 women; mean age, 53.6 years; range, 28 to 89 years) with no head and neck disease or laryngeal or pulmonary disorders.

Fiberscopic Examination. Physical and videofiberscopic examinations were performed with a flexible laryngoscope (Olympus, ENF type PIII, or Machida, ENT-30 type PIII) by the same otolaryngologist (S.P.) in all subjects. This videofiberscopy included a functional assessment of deglutition of dry swallows and swallowing of thick cream, methylene

blue water, and marshmallows.

Clinical Measurements of Acoustic and Aerodynamic Parameters. Aerodynamic and acoustic analyses were performed by a speech-language pathologist using an SQ Lab workstation and an EVA (French acronym for assisted voice evaluation) analyzer. All of the subjects were recorded in a quiet room. For acoustic analysis, a mouth-to-microphone distance of 30 cm was maintained during production of the vowel /a/ voiced at a comfortable pitch and loudness level. Acoustic parameters included measurements of jitter, shimmer, number of harmonics, and the aperiodic component of voice expressed by the "1-ratio signal."¹⁶ The number of harmonics and the 1-ratio signal were calculated from spectral analysis. For aerodynamic parameters, both oral and nasal airflows were assessed by separate airflow transducers. The following parameters were recorded: maximum phonation time (MPT) during production of a sustained /a/ (seconds), expired volume on MPT during production of /a/ and /s/ (liters), and flow rate measured during production of a sustained /a/ (liters per second). Expired and inspired volumes (liters) were calculated during the following sentence: "*C'est une affaire intéressante, qu'en pensez-vous?*" The expired volume on MPT during production of /a/ and /s/ (liters) expressed the maximum amount of expired air available either for a high-intensity phonatory production (requiring a small amount of air) evaluated on the vowel /a/, or for a low-intensity phonatory production (requiring a large amount of air) on the consonant /s/. The mean values of each measure were calculated from 3 consistent productions recorded for the flow rate, as well as expired and inspired volumes during the sentence, while the best performance among 3 trials was selected for parameters based on the MPT (MPT on a sustained /a/, and expired volume on MPT during /a/ and /s/ productions).

Questionnaire. The patient self-assessment was evaluated by a questionnaire especially designed for the study (of about 50 questions), related to the patient's subjective difficulties in phonation, swallowing, and breathing. The main items are reported in Table 1. It was especially focused on phonatory and respiratory efforts and on the impact on quality of life (family, social, and professional aspects). The responses were scored on a scale with a maximum of 3 points (0 for none or normal, 1 for slight alteration, 2 for marked alteration, and 3 for severe alteration) to evaluate vocal status, phonatory breath control, and glottic closure efforts. For respiratory function, breathiness during daily efforts and exercise, effective cough, and impact on the quality of life, responses were scored as yes or no (presence or absence of the complaint).

TABLE 1. RESULTS OF PATIENT SELF-ASSESSMENT

	Preoperative	Postoperative	p*
Vocal status			
Global quality†	2.3 ± 0.6	0.5 ± 0.3	1.2 × 10 ⁻⁸
Efficiency†	2.6 ± 0.6	0.3 ± 0.5	6.3 × 10 ⁻⁹
Strength†	2.6 ± 0.6	0.8 ± 0.6	9.3 × 10 ⁻⁶
Sonority†	2.6 ± 0.9	0.7 ± 0.6	4.0 × 10 ⁻⁵
Phonatory breath control			
Breathiness†	2.3 ± 1.2	0.2 ± 0.4	<.0001
Asthenia during phonation†	2.0 ± 1.4	0.3 ± 0.4	.0001
Global asthenia†	1.7 ± 1.5	0.2 ± 0.5	.0007
Strain†	1.9 ± 1.3	0.2 ± 0.4	.0003
Return to steady breathing†	1.6 ± 1.0	0.2 ± 0.4	.0001
Instability†	1.9 ± 1.4	0.8 ± 0.6	.006
Respiratory function			
Global disturbance‡	14	5	
Sensation of shortness of breath‡	12	1	
Deeper inspirations‡	10	3	
Increased respiratory frequency‡	7	1	
Disturbed inspiration‡	7	2	
Smaller inspired airway‡	6	2	
Breathiness during daily efforts			
Global‡	18	5	
Going upstairs‡	12	5	
Phonation‡	12	2	
Walking‡	6	1	
Breathiness during exercise			
Global‡	14	5	
Difficulty in getting one's breath‡	11	3	
Glottic closure effort†	0.6 ± 0.6	0.3 ± 0.5	.0015
Effective cough‡	7	15	
Impact on quality of life			
Global disturbance‡	16	2	
Disturbance in family life‡	9	0	
Disturbance in social life‡	9	0	
Disturbance in professional life‡	9	2	

*Statistically significant difference at p < .02 between preoperative and postoperative values.
†Mean values and standard deviations for responses evaluated on scale from 0 to 3.
‡Number of patients with responses scored as yes (presence of complaint).

Analysis of Evaluations. Evaluation of the voice was based on both acoustic parameters and the patient self-assessment. Phonatory breath control was evaluated by aerodynamic measures and by patient self-assessment, and respiratory function was evaluated by patient self-assessment. Mean values and standard deviations were determined from the results. Parameters were compared in terms of preoperative and postoperative values and to controls. Student's *t*-test was applied for statistical analysis, with a significance level of $p < .02$.

RESULTS

Fiberscopic Examination and Swallowing Evaluation. At preoperative physical and videofiberscopic examinations, the vocal fold was fixed in an inter-

mediate (12 patients) or paramedian (6 patients) position, resulting in incomplete laryngeal closure during phonation in all patients. The vocal fold showed marked atrophy in 11 cases. The pharynx was also paralyzed in 5 patients, with opened pyriform fossae and paralysis of the posterior pharyngeal wall. Aspiration of liquids was observed in 10 patients, and of thick cream in 6 patients. Postoperative videofiberscopic examinations revealed no recovery of vocal fold mobility. Medialization resulted in complete glottic closure during the production of a sustained /a/ in 15 patients, whereas a posterior glottal gap was still present in the other 3 (2 from autologous fat injection, 1 after thyroplasty). The postoperative videofiberscopic swallowing study showed that the aspiration of liquids had disappeared in all patients ex-

TABLE 2. MEAN ($\pm$ SD) VALUES OF ACOUSTIC PARAMETERS AND COMPARISON OF RESULTS BEFORE AND AFTER MEDIALIZATION

	Control (n = 14)	Preoperative (n = 18)	Postoperative (n = 18)		
			Short Term	Middle Term	Long Term
No. of harmonics	20 $\pm$ 8	7 $\pm$ 5	15 $\pm$ 7 (p = .0004)*	16 $\pm$ 16 (p = .022)	16 $\pm$ 8 (p = .008)*
1-Ratio signal	6.0 $\pm$ 10.0	39.6 $\pm$ 28.9	12.2 $\pm$ 10.0 (p = .001)*	30.4 $\pm$ 31.3 (p = .49)	23.7 $\pm$ 21.1 (p = .12)
Jitter (%)	0.5 $\pm$ 0.5	6.9 $\pm$ 7.9	1.4 $\pm$ 1.5 (p = .011)*	2.5 $\pm$ 5.0 (p = .29)	1.0 $\pm$ 0.6 (p = .007)*
Shimmer (%)	0.6 $\pm$ 0.8	1.8 $\pm$ 1.5	0.7 $\pm$ 0.3 (p = .011)*	2.5 $\pm$ 2.4 (p = .53)	0.8 $\pm$ 0.4 (p = .019)*

*Statistically significant difference at $p < .02$ between preoperative and postoperative values.

cept 1, in contrast to that of thick cream, which persisted in 3 patients.

On the questionnaire, aspiration of liquids was reported before operation in all patients, 4 of them also exhibiting blockage, aspiration of solids, and/or nasopharyngeal reflux. Aspiration of liquids disappeared in most of them after medialization (partly persisting in 1 patient), although caution during drinking was frequently reported during the ST follow-up. The blockage, aspiration of solids, and/or nasopharyngeal reflux persisted in the 4 patients after medialization. In addition, coughing was considered to be effective in 15 patients after medialization (in contrast to 7 before medialization).

Vocal Assessment. Dysphonia was exhibited before operation in all patients. On the questionnaire, the mean global quality of voice was significantly improved by medialization (on the 3-point-maximum scale, mean grade of 2.34 before operation and 0.53 after operation), as were efficiency, strength, and sonority (Table 1). Most of the patients were greatly satisfied by their medialization and said that they had recovered their voice. However, singing and loud speech were still disturbed in some of the patients, in contrast to daily conversation such as telephoning.

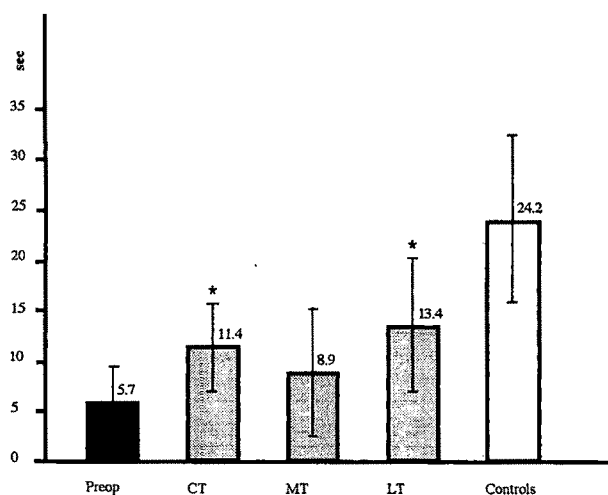


Fig 1. Mean values and standard deviations of maximum phonation time in seconds. Preop — preoperative, ST — short term, MT — middle term, LT — long term, * — statistically significant difference at $p < .02$ between preoperative and postoperative values.

The acoustic analysis (Table 2) demonstrated that all of the preoperative parameters were significantly disturbed as compared to the control population, and that most of them were improved after medialization. Doubling of the mean number of harmonics (from a preoperative value of 7 to a postoperative value of 16) was statistically significant at ST and LT after medialization; a diminished effect was observed at MT. Nevertheless, the mean values varied between sexes: values for female patients reached those of controls, whereas those for male patients were lower. The mean of the 1-ratio signal was improved at ST, but deterioration was observed at MT and LT, with high variations of these values. The mean jitter and shimmer rates had significantly decreased at ST and LT, with levels near normal. However, at MT, an increase of jitter and shimmer values was observed. Except for the 1-ratio signal, all of the acoustic parameters were improved after medialization and were statistically significant at ST and LT ($p < .02$).

Phonatory Breath Control Assessment. Self-assessment by the patients demonstrated that difficulties during phonation related to disturbance of breath control were improved. On the 3-point-maximum scale, the mean values of breathiness during phonation, asthenia during phonation, global asthenia, strain, return to steady breathing after phonation, and instability of voice were statistically decreased (Table 1).

All of the preoperative aerodynamic parameters were significantly disturbed in comparison to the control population. In addition, the results were not affected by the preoperative pulmonary function. (No difference was observed between patients with lung disease and the others.)

The mean MPT during production of a sustained /a/ increased significantly at ST ($p = .0006$) and LT ($p = .010$) after surgery: from 5.7 seconds to 11.4 and 13.4 seconds, respectively (Fig 1). There was a diminished effect at MT ($p = .21$). The results in women reached the values of the control subjects, in contrast to men, in whom the MPT was lower, so that the global MPT was lower than that of healthy subjects.

The mean results of the expired volume on MPT

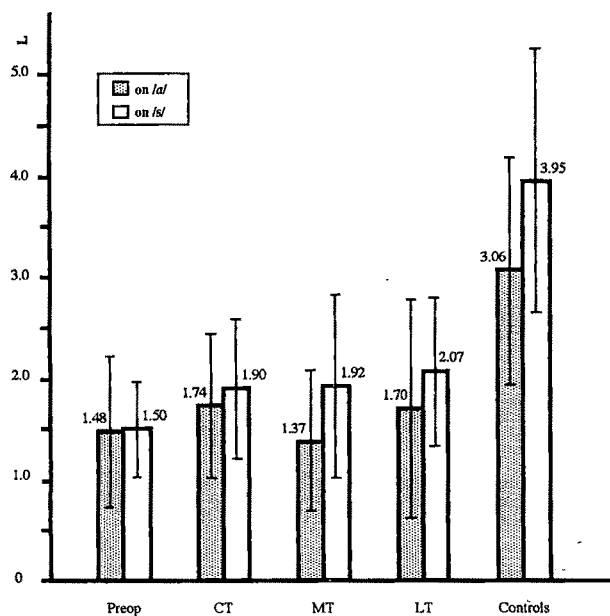


Fig 2. Mean values and standard deviations of expired volumes (liters) on maximum phonation time during production of /a/ and /s/. See legend of Fig 1.

during production of /a/ and /s/ showed a slight improvement that was not quantitatively significant ($p > .09$), although the volumes were qualitatively normalized (resulting in a higher expired volume used on /s/ than on /a/; Fig 2).

The mean flow rates measured during a sustained /a/ (Fig 3) decreased statistically after medialization at ST ($p = .004$) and LT ($p = .0012$), with a slightly diminished effect at MT ($p = .16$). The postoperative values at ST and LT reached those of controls.

The mean expired volume for the sentence "C'est une affaire intéressante, qu'en pensez-vous?" significantly decreased, and demonstrated a normalization both at ST ($p = .0008$) and LT ($p = .0003$) after the procedure (Fig 4). There was a diminished effect at

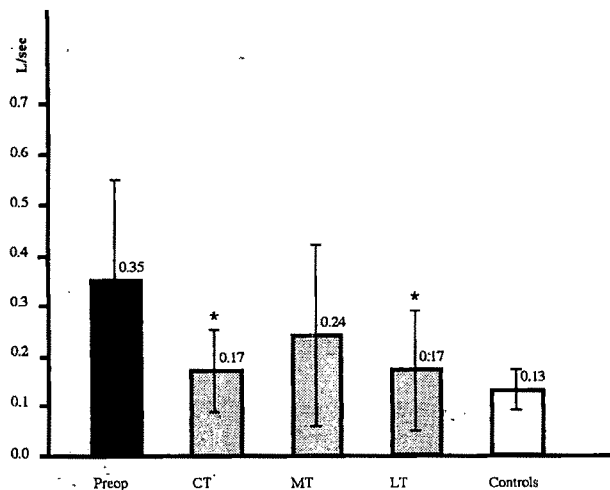


Fig 3. Mean values and standard deviations of flow rates (liters per second) on sustained /a/. See legend of Fig 1.

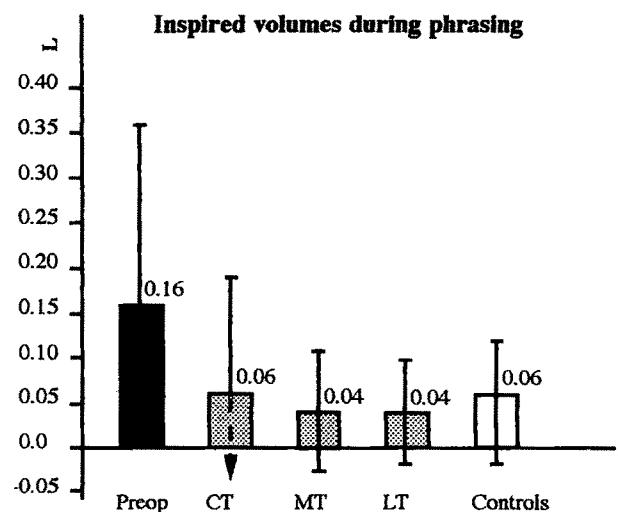
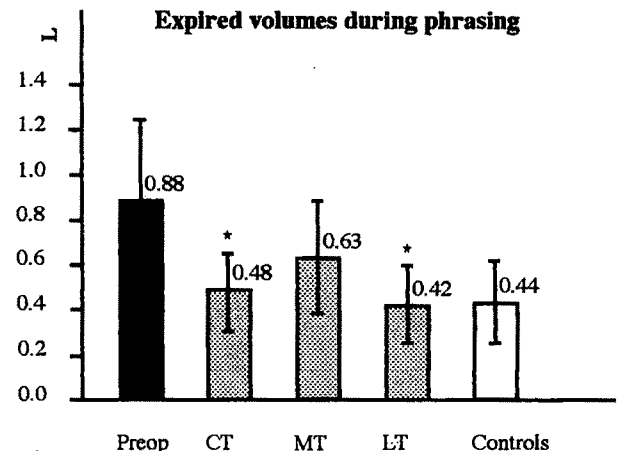


Fig 4. Mean values and standard deviations of expired and inspired volumes (liters) during utterance of "C'est une affaire intéressante, qu'en pensez-vous?" See legend of Fig 1.

MT ($p = .06$). The mean inspired volume for the same sentence decreased from 0.2 L (with an SD of 0.2 L) to 0.04 L, but the change was not statistically significant ($p > .49$; Fig 4).

Respiratory Function Assessment. Respiration was improved by medialization, since difficulties were reported in most of the patients before operation (14 cases) and disappeared in 9 of them after operation (Table 1). Most of the subjective complaints were related to a sensation of shortness of breath, the need to take deeper inspirations, and the need to increase the respiratory frequency. Some patients also pointed out that they had a disturbed inspiration and complained of a smaller airway. These difficulties were exhibited by patients before operation whatever their pulmonary status, and were improved after operation. Breathiness during daily efforts and exercise decreased after medialization, although some difficulty during exercise persisted in 5 of the 14 active patients. Efforts requiring firm glottic closure with elevated intra-abdominal pressure, such as lifting and

defecating, were also improved, and the number of patients who considered that coughing was effective increased after operation.

Impact on Quality of Life. The patient self-assessment (Table 1) showed that the global impact of the paralysis (on phonation, breathing, and swallowing) before medialization affected daily life in all patients except 2. The quality of their family, social, and professional lives was improved after operation. Some difficulties at work persisted in 2 patients, especially when they had to talk in public.

DISCUSSION

In the present study, we have shown that there is a disturbance of breath control in unilateral vocal fold paralysis, and that some degree of laryngeal obstruction, as a consequence of medialization, is required to improve both vocal status and breath control. This conclusion was demonstrated by a comparative analysis with a healthy population, and by the fact that medialization of the paralyzed vocal fold significantly improved the acoustic properties of the voice, as well as respiration. Our analysis was not based on pulmonary tests, but aerodynamic parameters were used to study phonatory breath control, and patient self-assessment was used to evaluate phonatory breath control and respiratory function before and after medialization. Swallowing and coughing were also studied, since aspiration and ineffective coughing may compromise the protective functions of the larynx and respiration. The constitution of a control population was necessary for us to study the preoperative status of patients and the effects of medialization. In this way, numeric values were obtained that allowed objective comparisons and statistical evaluation. This step is important, since the patient self-assessment demonstrated a significant improvement for most of parameters tested, although some of the instrumental measures did not reach normal values. This discrepancy has already been reported,¹⁷⁻²¹ and may be due to the fact that in patient self-assessment, improvement is always compared with a preoperative pathological status. In contrast, the criteria used in instrumental analyses are based on normal values from a healthy population.

Concerning phonatory breath control, this present study demonstrated, in comparison to controls, a marked disturbance prior to medialization, as shown by the results of the aerodynamic parameters that were not affected by the pulmonary status. It was well assessed by the values of MPT on a sustained /a/, which has been used in most of the recent studies independent of the medialization procedure that was used.^{14,15,20,22-26} This was associated with a decrease in the mean flow rate, as previously reported²²: for

the same expired volume, the duration of phonatory production increased, while the flow rate decreased. In addition, although the expired volume on MPT was not quantitatively normalized, medialization allowed a qualitative laryngeal control of the expired volumes required to produce either high-intensity (on /a/) or low-intensity (on /s/) phonatory activity. The fact that the expired volumes on MPT did not differ between these tasks before medialization, in contrast to the controls, should be related to the difficulty for a paralyzed vocal fold in producing high-intensity phonatory activity. It also indicates that the ability of the vocal fold to increase the subglottic pressure is more impaired during high-intensity phonatory activity like that required on /a/ than it is during the production of a consonant like /s/. During phrasing, which is not a situation of performance such as MPT, the values of the expired volume decreased significantly to reach those of controls. Medialization allowed the patient to reduce the expiratory effort of natural voice activity (eg, phrasing) more easily than that of a situation of performance. The inspired volume used during phrasing also decreased, although the high value of the standard deviation prevented any significant difference. The fact that the volumes measured were improved qualitatively (during MPT) or quantitatively (during phrasing) demonstrates that medialization does not compromise the airway for phonation, but successfully regulates the phonatory breath control. This finding is in accordance with the study of Leder and Sasaki,¹⁵ which demonstrated, after medialization, a reduction in the number of inspirations used during the oral reading of a text. In addition, in the present study, the results of the questionnaire showed that phonatory effort was greatly improved by medialization, in agreement with the aerodynamic parameters.

Concerning respiratory function, however, it has been well demonstrated by pulmonary aerodynamic parameters, especially on flow-volume loops, that some degree of airway obstruction occurs in unilateral vocal fold paralysis and compromises respiration.¹¹⁻¹⁴ Some controversy still remains as to whether medialization induces a new degree of obstruction. This controversy may result from the method of analysis used to define laryngeal obstruction, or the procedure of medialization that was performed. In Kashima's study,¹¹ an improvement in inspiratory flow rates occurred after Teflon injection. In contrast, Janas et al¹³ demonstrated an increased rate of laryngeal obstruction (from about 20% to 47% of patients) after thyroplasty, but the thyroplasty was combined in some cases with cricothyropexy and arytenoid adduction. In this earlier study, the evaluation of obstruction was based on the increased ratio of the ex-

pired flow rate to the inspired flow rate at 50% of the vital capacity, which was due to an increase in the inspired flow rate only. In a series of 6 patients in whom thyroplasty was performed, a reduction in both inspiratory airflow and glottic aperture under exercise conditions was demonstrated, but preoperative evaluation was not performed.¹² In the study of Saarinen et al,¹⁴ the only parameter that was significantly disturbed after autologous fascia medialization was a slight limitation of the mean peak inspiratory flow (ie, forced inspiration). The selective restriction of the inspiratory flow rate reported in most of these studies, with normal expiratory flow-volume variables, indicates a variable degree of upper airway obstruction. On the basis of these previous data and our results, in which the patients found an improvement in the control of respiratory function (sensation of shortness of breath, increased respiratory frequency, breathiness during daily efforts), a variable degree of laryngeal obstruction by medialization would improve, in addition to the vocal status and phonatory breath control, the respiratory function in unilateral vocal fold paralysis. Moreover, preoperative disturbance of respiratory function was especially reported on the patient questionnaire in terms of inspiration (deeper inspirations). This disturbance may be easily detectable, especially in listening to patients during phrasing, and in questioning them about the difficulties they encounter during exercise. These inspiratory efforts may be generated to increase the amount of air available, as there is a permanent air escape from the glottic aperture and a decrease in subglottic pressure. After medialization, we have demonstrated that regulation of the expired volume, by correcting the glottic escape, improved efforts that require firm glottic closure, and also allowed a decrease in the inspiratory effort. This would not be the case if the preoperative disturbance of breathing was caused by the obstruction of the paralyzed vocal fold, as in infants, since medialization tends to increase glottic obstruction. Consequently, the disturbance of the respiratory cycle in adults would be due to an overenlarged glottic aperture rather than to laryngeal obstruction. However, during measures of performance, such as exercise or forced inspiration, slight impairment of the airflow might occur. This should be partly dependent on the medialization procedure used and on the effort used by the patients. In this work, a comparison between the methods of medialization could not be made, because the number

of thyroplasties was too low. In the literature, subjective complaints of laryngeal obstruction are uncommon; airway compromise has been reported when medialization was combined with arytenoid cartilage manipulation,²⁷ and in active patients with an increased ventilatory demand.¹³ More investigations would be required to measure the impact of exercise on breath control by adapting respiratory tests.

This study also demonstrated 2 particular features: different results were obtained in women and men, and fluctuations were observed in the MT results. The better results observed in women were not correlated with age, pulmonary function, or the severity of the preoperative voice or aerodynamic impairment. This finding may be due to the smaller size of the female larynx, which may be more adapted to being closed by medialization, and/or to a different metabolism of fat. Stroboscopy, as well as magnetic resonance imaging,²⁸ could be performed to compare the sexes in terms of the dynamic properties of glottic closure and the fat volume in the muscles. Deteriorations in the MT results relative to the ST and LT results were also observed on both acoustic and aerodynamic analyses. The initial improvement was followed by a transient diminished effect, but the improvement remained stable after 7 months. This finding has been reported previously,²⁹ but was thought to be controversial.²² It may be that a readjustment is required to relearn a more natural voice and breath control, which may temporarily destabilize the results. The role of voice therapy during the period of diminished effect should be investigated. In addition, for the best evaluation of the success of medialization, studies should be performed either within 3 months of the operation or 7 months or more after the operation.

In conclusion, acoustic and aerodynamic analyses, as well as patient self-assessment, demonstrate that disturbances of phonatory breath control and respiratory function in adult unilateral vocal fold paralysis due to airflow escape are improved by medialization. This procedure provides a more natural breath control, allowing reduced vocal effort, although some difficulty during exercise may persist. This study also shows that an evaluation of the outcome of medialization may consider breath control as a parameter of laryngeal function, in addition to vocal status, especially in patients with lung dysfunction either from surgery or from aspiration.

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OTOLARYNGOLOGICAL SURGERY AND UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN: AN EPIDEMIOLOGICAL STUDY

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The objective of the study was to assess the occurrence of different procedures of upper airway surgery and estimate their relationship to specific upper respiratory tract infections and constitutional factors. In a population-based cross-sectional study in Oslo, Norway, of 3,763 preschool children 3 to 4 years of age, the otolaryngological surgeries adenoidectomy, tonsillectomy, myringotomy, ventilation tube insertion, and combinations of these were the outcome measures. The results showed that by 4 years of age, 13% (n = 501) had undergone operation, and approximately two thirds of the operations involved middle ear surgery. Although surgery was related to the occurrence of upper respiratory tract infections, the type of surgery was not related to the specific infection. In the children with operations, the occurrence of recurrent otitis media (≥ 3 infections in the previous 12 months) was almost fivefold higher than in children without operations (adjusted odds ratio [OR_{adj}] = 5.19 [3.15 to 8.54]). A low level of maternal education (OR_{adj} = 1.61 [1.05 to 2.7] compared to the group with a high level of education) and atopy on the part of the child (OR_{adj} = 1.58 [1.20 to 2.07]) increased the probability for upper airway surgery independently of the experience of infections. In conclusion, early pediatric otolaryngological surgery is common. The decisions for surgical treatment vary substantially and are not closely related to the specific infections. The influence of other factors such as maternal education indicates that decisions for surgery are not entirely based on medical evidence.

KEY WORDS — epidemiology, guideline, otitis media, surgery, upper respiratory tract infection.

INTRODUCTION

Upper respiratory tract infections are common in children, and treatment is both medical and surgical.^{1,2} Correspondingly, surgery in the upper airways is the most common operation in childhood.^{2,3} Upper airway surgery includes adenoidectomy, tonsillectomy, myringotomy, ventilation tube insertion, and combinations of these. Different types of surgical procedures have evolved over the past 30 years, but their efficacy has not been assessed thoroughly. Only recently have controlled clinical trials been conducted to evaluate the specific surgical procedures.⁴ To date, the guidelines and clinical treatment recommendations for recurrent upper respiratory tract infections are nonspecific.^{1,2,5}

In the decision-making for surgery, a dilemma is to balance between unnecessary operations and withholding operations from children who would benefit from surgery. To optimize surgical practice, it is necessary to know about the prevailing practice. To our knowledge, there are no population-based reports of the occurrence of otolaryngological surgery in early childhood. The present study elaborated pediatric surgical practice for upper respiratory tract morbidity in 4-year-old children of Oslo, Norway. We assessed the occurrence of different procedures and the rela-

tionship of the type of surgery to the type of infection and to constitutional and socioeconomic factors.

MATERIALS AND METHODS

Study Population and Data Collection. The source population included children born in Oslo in 1992 who were official residents of the city by November 1996. Because of expected language difficulties in providing information by self-administered questionnaires, children of non-Norwegian parents were excluded from the retrospective study (n = 1,398). The 4,863 of the 4,966 families who fulfilled the inclusion criteria (98%) and for whom the current address was available received self-administered questionnaires on their history of upper airway surgery and health and familial characteristics. The number of respondents was 3,853 (79%). The exclusion of 90 children whose parents did not respond to the question on upper respiratory tract surgery left 3,763 children for the current analyses. The study population is more thoroughly described elsewhere.⁶

Outcome Measures and Determinants. The outcomes of interest were the upper airway surgeries adenoidectomy, tonsillectomy, myringotomy, ventilation tube insertion, and any combination of these. In this study, middle ear surgery was limited to out-

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TABLE 1. CHARACTERISTICS OF NORWEGIAN PRESCHOOL COHORT BY OTOLARYNGOLOGICAL SURGERY

	Upper Airway Surgery (13%; n = 501)		No Surgery (87%; n = 3,262)		Entire Cohort (N = 3,763)	
	No.	%	No.	%	No.	%
Gender (male)	281	56	1,653	51	1,934	51
Birth weight ($\leq 2,500$ g; 244 missing)	34	7	154	5	188	5
Maternal education (33 missing)						
<12 y	45	9	213	7	258	7
12-15 y	346	70	2,113	65	2,459	66
>15 y	104	21	909	28	1,013	27
Single parenthood (6 missing)	92	18	541	17	633	17
Siblings (yes; 245 missing)	243	51	1,608	53	1,851	49
Atopic disease* (71 missing)	107	22	375	12	482	13
Maternal smoking in past 12 mo (103 missing)	188	38	1,001	32	1,189	33
Paternal smoking in past 12 mo (258 missing)	152	32	916	30	1,068	31
Duration of breast-feeding (≤ 3 mo; 205 missing)	86	19	419	14	505	14
Day-care arrangement						
Home care	41	8	329	10	370	10
Family day care	8	2	66	2	74	2
Day-care center	424	85	2,591	79	3,015	80
Mixed home care and day-care center care	28	6	276	9	304	8

*Asthma, allergic conjunctivitis, or rhinitis.

patient procedures including myringotomy with or without ventilation tube insertion. Surgery including adenoidectomy or tonsillectomy was regarded as pharyngeal surgery. The combined-surgery group consisted of children who underwent both tonsillectomy and middle ear surgery.

We studied 3 types of determinants: 1) respiratory infections, 2) constitutional factors, and 3) socioeconomic factors. We focused on respiratory infections from the previous 12 months, including acute otitis media, tonsillitis or pharyngitis, common cold, chronic rhinitis, bronchitis, and pneumonia. Chronic rhinitis was defined as daily or weekly nasal secretion without coexistent common cold. Recurrent acute otitis media and tonsillitis or pharyngitis was defined as 3 or more infections during the past 12 months, and recurrent common cold was defined as 4 or more colds in the same period of time. Recurrent lower respiratory tract infections were defined as 3 episodes of bronchitis or 2 or more episodes of pneumonia in the past year. Lifetime recurrent acute otitis media and croup was defined as 4 or more episodes of acute otitis media and acute laryngitis, respectively, during any 12-month period.

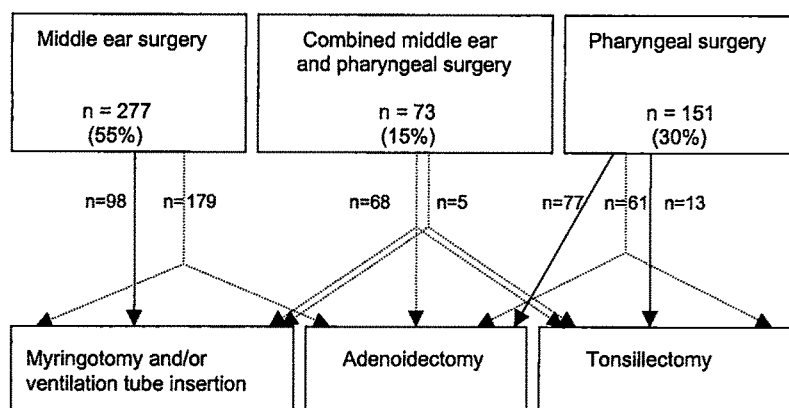
The constitutional factors included gender, birth weight, and atopic disease. Parental education was used as an indicator of socioeconomic status. Environmental covariates were parental smoking, number of siblings, and type of day care (as sources of exposure to infectious agents). The type of day care was divided into 3 categories: home care (at home with mother or father; at home with a nursing assistant; at

home but attending organized playgrounds together with other children); family day care (in a family or private nursery outside the home); and day-care center (owned by the municipality or privately owned). For this study, atopic disease was defined as any experience of physician-diagnosed asthma, allergic conjunctivitis, or rhinitis. The determinants and their distributions are listed in Table 1.

Statistical Analysis. We estimated the prevalence of surgeries and recurrent respiratory infections and compared the prevalence of infections between children who underwent different types of surgery to those who did not have surgery. The odds ratio (OR) was used as a measure of association between the occurrence of pharyngeal and/or middle ear surgery and the studied infections and other determinants. The adjusted OR (OR_{adj}) was estimated in logistic regression analysis with SPSS 8.0, with the covariates in Table 1 controlled for. On the basis of previous knowledge, 3 core covariates (gender, birth weight, and maternal education) were always included in the model. The other covariates were included if their inclusion had an impact on the accuracy and precision of the estimate of the studied relationship. We used SPSS 8.0 for all analyses.

RESULTS

Study Population. Of the study participants, the majority of children belonged to 2-parent families (83%) and had parents with more than 12 years of education. The median age was 4.3 years (SD, 0.3 year). Of all children, 12% had physician-diagnosed



Combinations of middle ear and pharyngeal surgical procedures among children in cohort. Surgery was performed in 13% of children (N = 501).

lifetime asthma, allergic conjunctivitis, or rhinitis. Ten percent of the children were cared for at home, 2% in private homes, 80% in day-care centers, and 8% in combined (home and center) day-care arrangements. The characteristics of the study population for the surgery and nonsurgery subgroups in Table 1 show that upper airway surgery was significantly more commonly performed in boys, in children with concurrent atopic disease, in children breast-fed for less than 3 months, and in children whose mothers had more than 15 years of education.

Occurrence of Surgery. Of the 13% of children in the study who underwent surgery (n = 501), 9% (n = 350) had middle ear surgery and 6% (n = 224) had tonsillectomy. The proportion of boys who underwent surgery was 14% (95% confidence interval [CI], 12% to 16%), in contrast to 12% of girls (95% CI, 10% to 13%). Of all 501 children who underwent operations, combinations of middle ear surgery were performed in 277 (55%), pharyngeal surgery in 151 (30%), and a combination of middle ear and pharyngeal surgery in the remaining 73 (15%). The Figure shows the numbers of children who underwent the different types of surgery and the corresponding combinations of

operative treatments.

Respiratory Infections as Determinants of Surgery. Table 2 shows the prevalence of recurrent respiratory infections by the different types of surgery. Approximately 30% of the children experienced 3 or more episodes of common cold during the past 12 months. The prevalences of chronic rhinitis, recurrent common cold, and acute otitis media were higher in all surgery subgroups than in the nonsurgery group. Between the surgery subgroups, however, there were only small differences (Table 2). Furthermore, otitis media and pharyngitis or tonsillitis were not significantly more frequent in children who underwent middle ear and pharyngeal surgery. Only otitis-proneness was more common in the middle ear surgery group and in children who underwent combined middle ear and pharyngeal surgery (Table 2).

Table 3 gives the proportion of children who underwent surgery according to each of the recurrent infections and crude and adjusted ORs for the relationship between the experience of respiratory infections and risk for surgery. The adjustment was first made for the other respiratory infections, then with control for

TABLE 2. PREVALENCE RATES OF RECURRENT RESPIRATORY INFECTIONS IN PRESCHOOL CHILDREN BY TYPE OF UPPER RESPIRATORY TRACT SURGERY

	Middle Ear Surgery (n = 277)		Pharyngeal Surgery (n = 151)		Combined Middle Ear and Pharyngeal Surgery (n = 73)		No Surgery (n = 3,262)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Recurrent respiratory infections in past year								
Chronic rhinitis (daily or weekly in past year)	4	2-7	10	5-15	11	4-18	3	2-2
Recurrent common cold (≥ 3 in past year)	41	35-47	41	33-49	39	28-50	27	25-28
Recurrent acute otitis media (≥ 3 in past year)	10	6-13	6	2-10	10	3-13	1	1-2
Recurrent pharyngitis or tonsillitis (≥ 3 in past year)	3	1-5	5	2-9	1	0-4	1	0-2
Recurrent lower respiratory tract infections (≥ 3 bronchitis or ≥ 3 episodes of pneumonia in past year)	7	4-10	6	2-10	7	1-13	3	3-4
Recurrent respiratory infections in lifetime								
Recurrent acute otitis media (≥ 4 in 12 months)	57	51-63	30	23-37	51	40-63	8	7-8
Recurrent croup (≥ 4 in 12 months)	7	4-10	10	5-14	10	3-17	4	3-4

TABLE 3. PROPORTION, CRUDE ODDS RATIO, AND ADJUSTED ODDS RATIOS WITH 95% CONFIDENCE INTERVALS FOR UPPER AIRWAY SURGERY AMONG PRESCHOOL CHILDREN BY VARIOUS RESPIRATORY INFECTIONS

	No. of Children in Each Category	Proportion of Children Operated On	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio* (95% CI)	Adjusted Odds Ratio† (95% CI)
Recurrent acute otitis media					
Two or fewer in past year	3,391	0.11	Reference	Reference	Reference
Three or more in past year	362	0.35	7.99 (5.08-12.60)	6.12 (3.80-9.86)	5.19 (3.15-8.54)
Recurrent pharyngitis or tonsillitis					
Two or fewer in past year	3,485	0.12	Reference	Reference	Reference
Three or more in past year	268	0.26	3.25 (1.84-5.76)	1.88 (0.98-3.60)	1.74 (0.90-3.38)
Recurrent common cold					
Three or fewer in past year	1,805	0.11	Reference	Reference	Reference
Four or more in past year	1,948	0.16	1.85 (1.52-2.24)	1.61 (1.32-1.98)	1.59 (1.28-1.97)
Chronic rhinitis (28 missing)					
Less than weekly in past year	3,598	0.03	Reference	Reference	Reference
Daily or weekly in past year	127	0.28	2.61 (1.76-3.92)	2.35 (1.55-3.58)	2.16 (1.41-3.43)

Reference category is children without recurrent infections. Note: information on health outcomes is missing in 19 to 59 cases.

*Adjusted odds ratios as controlled for respective respiratory infections in Table.

†Adjusted odds ratios as controlled for gender, birth weight, maternal education, atopic disease, recurrent acute otitis media, recurrent tonsillitis or pharyngitis, recurrent common cold, and rhinitis.

constitutional and socioeconomic factors. The risk for surgery was higher in children who experienced recurrent acute otitis media (crude OR = 7.99; 95% CI, 5.08 to 12.60), pharyngitis or tonsillitis (crude OR = 3.25; 95% CI, 1.84 to 5.76), common cold (crude OR = 1.85; 95% CI, 1.52 to 2.24), or chronic rhinitis (crude OR = 2.61; 95% CI, 1.76 to 3.92). Table 3 shows a weakened effect of otitis media when controlling for other infections (6.12; 95% CI, 3.80 to 9.86), indicating an independent effect of the number of infections on surgery.

Constitutional and Socioeconomic Determinants of Surgery. The relationships of the risk of surgery to constitutional and socioeconomic factors are shown in Table 4. The risk of surgery was higher in boys, atopic children, and low-birth weight children. When other infections were adjusted for, only the effect of atopy remained (OR_{adj} = 1.58 [95% CI, 1.20 to 2.07]). A low level of maternal education was also a strong determinant of surgery. Compared to the reference category of 15 years or more, the OR_{adj} of surgery was 1.38 (95% CI, 1.08 to 1.77) for 12 to 15

TABLE 4. PROPORTION, CRUDE ODDS RATIO, AND ADJUSTED ODDS RATIOS WITH 95% CONFIDENCE INTERVALS FOR UPPER AIRWAY SURGERY AMONG PRESCHOOL CHILDREN BY CONSTITUTIONAL AND SOCIOECONOMIC FACTORS

	No. of Children in Each Category	Proportion of Children Operated On	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio* (95% CI)	Adjusted Odds Ratio† (95% CI)
Gender					
Male	1,934	0.14	1.24 (1.03-1.50)	1.21 (0.99-1.48)	1.18 (0.96-1.45)
Female	1,829	0.12	Reference	Reference	Reference
Birth weight					
≤2,500 g	188	0.18	1.45 (1.00-2.14)	1.40 (0.94-2.08)	1.31 (0.87-1.98)
>2,500 g	3,331	0.13	Reference	Reference	Reference
Atopic disease					
Yes	482	0.12	2.12 (1.67-2.69)	2.01 (1.56-2.58)	1.58 (1.20-2.07)
No	3,210	0.22	Reference	Reference	Reference
Maternal education					
<12 y	258	0.17	1.85 (1.26-2.70)	1.60 (1.06-2.41)	1.61 (1.05-2.47)
12-15 y	2,459	0.14	1.43 (1.13-1.81)	1.36 (1.07-1.77)	1.38 (1.08-1.77)
>15 y	1,013	0.10	Reference	Reference	Reference

Note: information on health outcomes is missing in 15 to 59 cases.

*Adjusted odds ratios as controlled for gender, birth weight, maternal education, atopic disease.

†Adjusted odds ratios as controlled for gender, birth weight, maternal education, atopic disease, recurrent acute otitis media, recurrent tonsillitis or pharyngitis, recurrent common cold, and rhinitis.

years of education, and 1.61 (95% CI, 1.05 to 2.47) for the low-education category.

DISCUSSION

In our population-based cross-sectional study, we found that upper airway surgery in Oslo is common during the first 4 years of life. Upper respiratory tract infections strongly determine surgical practice, but there seems to be substantial variation in the choice of procedure as a response to different types of infection. In addition, both the child's physical characteristics, such as atopy and low birth weight, and maternal education were determinants for subsequent surgery.

The cross-sectional study design allows a valid estimation of the occurrence of surgery in the study population, but there may be some limitations in the inference from the associations between infections during the past year and surgery. The main concern is the timing of surgery. If the surgery took place more than 1 year earlier than the data collection, it could have influenced the occurrence of infections during the past year. Most likely, however, the majority of children underwent surgery within 12 months of the data collection. In a previous study, the mean age for pediatric surgery in Oslo hospitals was as high as 4½ years, and only 25% of all children who underwent surgery did so before 34 months of age, the average age for the retrospective report of infections in our study.⁷

Upper respiratory tract infections are common in children, and treatment recommendations include both medical and surgical interventions.^{1,2} Consequently, otolaryngological procedures are the most common type of surgery in childhood.^{3,5} In the decision-making for surgery, a dilemma is to balance between unnecessary operations and withholding operations from children who would benefit from surgery. To optimize surgical practice, it is necessary to know about the prevailing practice. To our knowledge, the present study is the first comprehensive report of the occurrence of otolaryngological surgery in early childhood in any population. Our population-based estimate of upper airway operations in 13% of children confirms that individualized surgical treatment of respiratory infections is common. Kogan et al⁸ reported a prevalence of 7% for ventilation tubes in a sample of 3-year-old US children, but did not give figures for other surgical procedures in the upper airways.

Different types of surgical procedures for upper airway infections have evolved over the past 30 years in clinical practice. The usefulness of different procedures in various respiratory problems has not been

studied systematically; hence, the indications for pharyngeal and middle ear surgery have fluctuated.^{3,9} The guidelines have allowed a substantial choice of treatment according to the physician's individual preference.^{1,2} The variation in type of surgical treatment in our study reflects the current controversy and uncertainty concerning the indications for these procedures.⁵ For example, although short-term benefits of myringotomy have been shown in the treatment of otitis media, the long-term benefits and complications deserve further study.¹⁰ Further, there is little supportive evidence of the efficacy of adenoidectomy for otitis media in younger children.⁴

The finding that the type of upper respiratory tract infection did not specifically predict the type of surgery suggests that factors other than infectious symptoms influence physician practice.¹⁰ This is consistent with a study from the United States that showed that other factors than set criteria influence the decision for ventilation tube insertion. Physicians reviewing the indications for tympanostomy tubes were in favor of surgery in as many as 79% of the cases, when only 29% of the cases supported the criteria set for surgical treatment.¹¹ Another study among US children found that differences in who receives ventilation tubes are partly explained by type of health insurance and low birth weight.⁸ Correspondingly, in our study there was a tendency for low birth weight to increase the probability of surgery. Interestingly, in the Norwegian public health care system, the level of education, rather than the wealth of the parents, seems to influence the probability of surgery. We found it striking that children of mothers with a low level of education and probably of lower socioeconomic status are more likely to undergo surgery.

It is known that upper respiratory tract infections are common in childhood and that the incidence declines with age.¹²⁻¹⁴ In our study, outpatient middle ear and pharyngeal operations were significantly related to acute otitis media, common colds, and chronic rhinitis. Recurrent otitis media, the most common cause for upper airway surgery,^{15,16} increased the probability for surgery fivefold. Our results confirm that children with infections undergo surgery. Furthermore, the similarity in prevalence of respiratory infections between the pharyngeal and middle ear surgery subgroups suggests that we tend to identify children who are predisposed to nonspecific infections, rather than children with a specific recurrent respiratory infection.^{12,17,18} Consequently, the type of upper respiratory tract infection does not seem to be related to one specific surgical procedure or specific surgical combination. Atopy was a determinant of surgery even after adjustment for infections. This

result could be explained by poor adjustment for infections, but it may indicate that atopic children in general are more readily submitted to surgery.

The present results indicate that decisions for the most common surgery in childhood vary substantially and are not closely related to specific infections. The influence of other factors, such as maternal education, indicates that the decisions may not be based on medical evidence. This finding emphasizes the need for information on the current clinical practice and further study of the efficacy of different surgical procedures in different types of clinical problems.

CONCLUSION

Early pediatric otolaryngological surgery is common; by 4 years of age, 13% of children have undergone surgery, and approximately two thirds of these operations involve middle ear surgery. In spite of existing treatment guidelines for middle ear and pharyngeal disease, combinations of surgical treatments are numerous. Respiratory infections and atopic disease are determinants of upper respiratory tract surgery. Furthermore, children are at increased risk for pediatric surgery in families with a lower level of maternal education.

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ATRIAL NATRIURETIC PEPTIDE RECEPTOR UPREGULATION IN THE RAT INNER EAR

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The purpose of this study was to further examine whether fluid homeostasis in the endolymphatic system could be regulated by a locally effective paracrine system involving atrial natriuretic peptides (ANPs) and their receptors. We assessed the biologic activity of the 3 ANP receptors (ANP-A, ANP-B, ANP-C) in the rat inner ear by measuring receptor upregulation after inner ear administration of ANPs. After appropriate anesthesia, female Lewis rats were injected with ANP via the round window. The animals were sacrificed 24 hours later, and RNA was isolated for reverse transcription–polymerase chain reaction (RT-PCR). Electrophoresis of RT-PCR products showed the presence of all 3 ANP receptor genes in both injected and control animals. Gene expression was significantly higher 24 hours after injection. These findings demonstrate that ANP receptors in the inner ear can be upregulated after injection of ANPs.

KEY WORDS — ANP receptor, atrial natriuretic peptide, endolymphatic sac, Meniere's disease, receptor upregulation.

INTRODUCTION

Dysfunction of the endolymphatic sac (ELS) is thought to be the underlying cause of the endolymphatic hydrops associated with Meniere's disease, which has no accepted cure.¹ It is believed that endolymph is produced by active transport of electrolytes in the stria vascularis of the cochlea and the dark cell epithelium of the vestibular organ, and that it is subsequently reabsorbed by the ELS.¹ The ELS epithelium synthesizes and secretes certain complex molecules, including hyaluronan, into the ELS lumen and is postulated to be involved in the sensitive regulation of endolymphatic volume and pressure.²

Recent research has suggested a possible role of atrial natriuretic peptides (ANPs) in the regulation of inner ear fluid.³⁻⁵ The ANPs are a group of hormones originally identified in mammalian atrial cardiocytes in the early 1980s.⁶⁻⁸ They are known to exert a smooth muscle effect, with vasodilatation, diuretic, and natriuretic activities in the kidney. Natriuretic peptides (ANP, brain natriuretic peptide [BNP], and C-type natriuretic peptide [CNP]) have been located in other extracardiac sites, such as the adrenal cortex, lung parenchyma, gallbladder, ciliary body of the eye, and choroid plexus of the brain.⁹⁻¹³ More recently, ANP receptors have been demonstrated in the inner ear of the guinea pig, with ANP-like activity located in the cochlea, the secretory epithelium of

the vestibular organ, and the ELS.³⁻⁵

In a separate study, we were able to demonstrate ANP receptor subclasses ANP-B and ANP-C in the epithelium of human ELS tissue using immunoperoxidase techniques. These findings were confirmed by reverse transcription–polymerase chain reaction (RT-PCR) techniques in the human tissue.¹⁴ The presence of these receptors suggests that the inner ear may have a paracrine or autocrine activity for the regulation of labyrinthine fluid and electrolytes. The purpose of the present study was to assess the biological activity of the 3 ANP receptors (ANP-A, ANP-B, ANP-C) in the rat inner ear by measuring receptor upregulation after inner ear administration of ANPs.

MATERIALS AND METHODS

Twelve female Lewis rats, 7 to 10 weeks old and weighing approximately 200 g, were injected with 2 μ L of ANP (rat atrial natriuretic factor [rANF], 28 amino acids, Peninsula Laboratories, Inc, San Carlos, California) dissolved in phosphate-buffered saline solution at a concentration of 500 ng/ μ L. The animals were anesthetized with 200 mg/kg sodium pentobarbital, and through a postauricular incision, the mastoid bulla was opened and the round window exposed under the operating microscope. By means of a 30- μ L syringe and 28-gauge needle bent at a 45° angle,

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the ANP was injected into the round window on the morning of day 1. The contralateral ear was not injected. The animals were then painlessly sacrificed the morning of day 2 (24 hours after injection). The otic capsule, including the ELS, and other organs (heart, liver, muscle, kidney, brain, lung, and uterus) were harvested and snap-frozen in liquid nitrogen. A similar procedure was performed on 12 control animals that had not received ANP injection. Kidney tissue was used as a positive control.

To isolate RNA for RT-PCR, the harvested otic capsule and ELS tissue were retrieved from the liquid nitrogen and homogenized with a Polytron. TRIzol reagent was added at a concentration of 1 mL per 100 to 200 mg tissue (TRIzol Reagent, Life Technologies, Gibco BRL, Rockville, Maryland), and the material was spun at 14,000 rpm for 10 minutes. The supernatant was transferred to a new tube, mixed with 200 μ L chloroform, and spun. The supernatant was then isolated and mixed with 500 μ L of 2-propanol. The material was cooled to -20°C for 30 minutes, then spun at 14,000 rpm for 20 minutes. The pellet was washed twice with 75% ethanol, then dissolved in 20 to 100 μ L diethyl pyrocarbonate-water (DEPC-H₂O). The optical density was measured at 260 nm to obtain the RNA concentration.

The RNA was then purified by DNase treatment. A total of 30 μ g RNA in 51 μ L DEPC-H₂O was mixed with 10 $\times$ DNase I buffer (6 μ L) and 1 unit/ μ L DNase I (3 μ L; Clontech, Palo Alto, California) and incubated at 37°C for 30 minutes. To this was added 10 $\times$ termination buffer (6 μ L), followed by 66 μ L phenol:chloroform (1:1). The solution was spun at 14,000 rpm for 10 minutes. The supernatant was isolated, mixed with 66 μ L chloroform, and spun at 14,000 rpm for 10 minutes. The top aqueous layer was transferred and mixed with 1/10 volume of 3 mol/L sodium acetate and 2 volumes of ethanol and cooled for 30 minutes on ice. The mixture was spun, and the pellet was washed and then dissolved in DEPC-H₂O. This yielded 10 to 20 μ g of purified RNA.

We performed RT-PCR using the reverse transcription procedure from Amersham Pharmacia Biotech (Piscataway, New Jersey, "First-Strand cDNA Synthesis"). Briefly, a total of 5 μ g RNA in 33 μ L of DEPC-H₂O was heated at 65°C for 10 minutes and then chilled on ice for 2 minutes. The RNA solution was transferred to a tube containing First-Strand Reaction Mix Beads; 2 μ L of random primer was added, and the contents were mixed by gentle vortex, then incubated at 37°C for 60 minutes. The total volume of first-strand complementary DNA (cDNA) was 35 μ L. Using the cDNA as a template, we designed the

primers of ANP receptors A, B, and C as follows: ANP-A receptor (length, 454 bp): sense 5'-GAG-AACAGCAGCAACATCCT-3', antisense 5'-AGC-GCACAGCATCCAGTAGT-3'; ANP-B receptor (403 bp): sense 5'-GGAAAACTGGTGGAGGAGC-3', antisense 5'-AATGCGGAAGGAAGAAAC-TG-3'; ANP-C receptor (258 bp): sense 5'-GCG-AAGCCTGAGTTTGAGAA-3', antisense 5'-ATACCGATCCCCATTAGCAT-3'; and reduced glyceraldehyde-phosphate dehydrogenase (345 bp): sense 5'-AGGCTGAGAATGGGAAG-3', antisense 5'-AGTACTCGGGAAGGTGC-3'.

The PCR reaction conditions were as follows: denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute. The PCR mixture was incubated for 10 minutes at 72°C after the amplification cycles, then stored at 4°C or used immediately for electrophoresis.

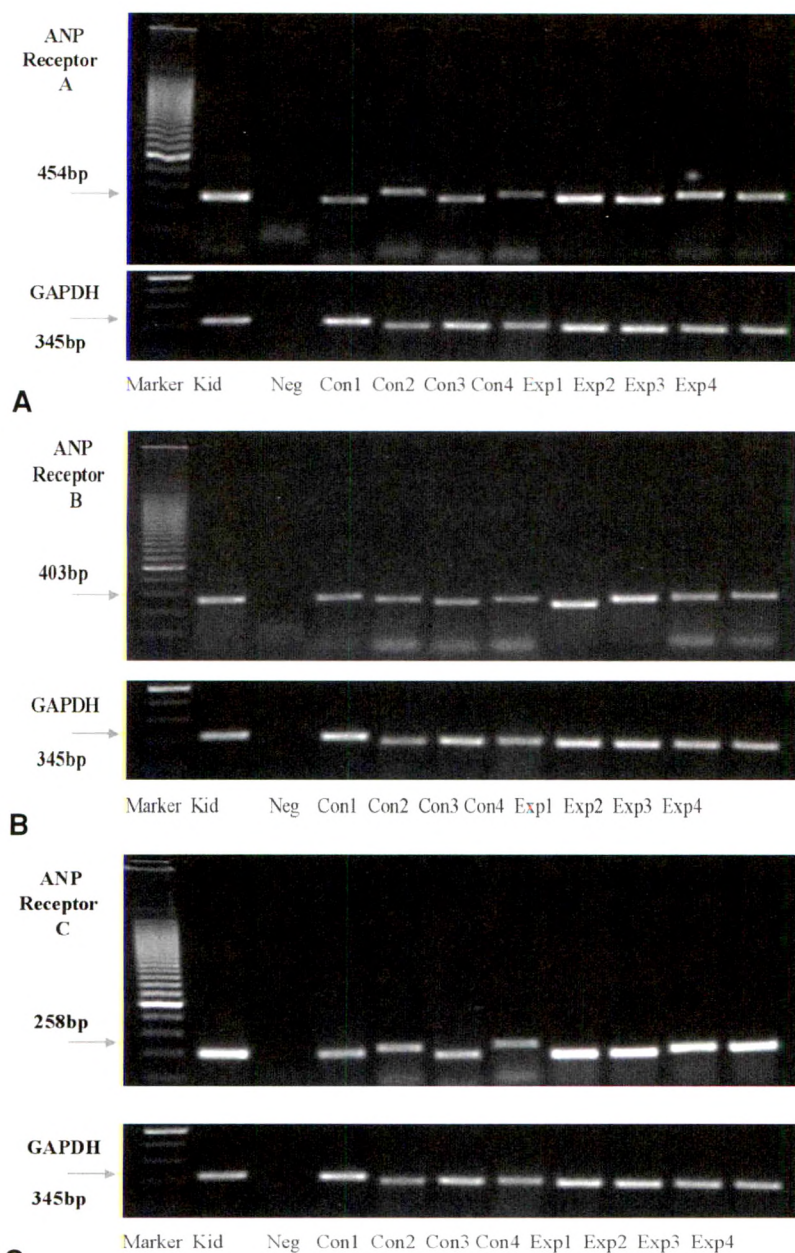
RESULTS

All 3 ANP receptor genes were present in the inner ears of both injected and control animals (see Figure), and the expression of the 3 ANP receptors was significantly higher 24 hours after ANP injection. Quantitative expression was performed with a densitometer. In order to determine the relative level of expression for each receptor subclass, we used kidney as the positive control for comparison. The average relative expression after injection was 90.3% for ANP-C (range, 86% to 99%), 84% for ANP-A (range, 83% to 88%), 76.5% for ANP-B (range, 68% to 87%), and 53.5% for noninjected controls (average of all receptor subclasses; range, 52.3% to 56%).

DISCUSSION

Recent studies have shown that ANPs and ANP-like receptors exist in the inner ear. Their involvement with fluid homeostasis in other organ systems would indicate that they could be involved in normal inner ear fluid regulation. Our current understanding of natriuretic peptides; their specific receptors; and their natriuretic, diuretic, and vasodilator effects is the result of numerous studies already performed on cardiac and renal tissue.^{6-8,15}

Three types of natriuretic peptide receptors have been identified and characterized. Two of these, ANP-A and ANP-B, represent the bioactive receptors.^{16,17} They consist of an extracellular domain for natriuretic peptide binding, a single transmembrane domain, a single ATP-binding domain for natriuretic peptide binding, and a guanylyl cyclase moiety.¹⁸ By activating guanylate cyclase, intracellular cyclic guanosine monophosphate concentrations increase and serve as a second messenger at the cellular level in various



Receptor expression at 24 hours for A) ANP-A, B) ANP-B, and C) ANP-C genes in inner ears of control (Con 1-4) and injected animals (Exp 1-4). GAPDH — reduced glyceraldehyde-phosphate dehydrogenase, Kid — kidney.

C

target tissues.¹⁹ Recently, a third receptor, ANP-C, was identified.²⁰ ANP-C, which is not coupled to guanylate cyclase, is thought to be biologically silent and serve as a specific clearance binding site for natriuretic peptides.²¹ The 3 natriuretic peptides have been shown to exhibit different binding affinities to the 3 receptor subtypes.²² ANP-A receptors preferentially bind ANP over BNP, with little reactivity for CNP. ANP-B receptors appear to be highly selective for CNP, with an affinity that is 50- to 500-fold higher than for ANP or BNP. ANP-C receptors bind all 3 natriuretic peptides, but with a rank order of ANP > BNP > CNP.

On the basis of the knowledge that natriuretic peptides are located within extracardiac tissues known

to play a role in the maintenance of fluid-electrolyte homeostasis (ie, ciliary process of the eye, choroid plexus of the cerebral ventricles), it has been postulated that they may also be present in the inner ear and have a similar activity. Lamprecht and Meyer zum Gottesberge³ and Meyer zum Gottesberge et al⁴ demonstrated the presence and localization of ANP receptors within the inner ear of the guinea pig. By means of autoradiographic techniques with radiolabeled ANP/CDD (atrial natriuretic peptide/cardioidilantin), receptors were localized to the stria vascularis, the organ of Corti, spiral ganglion cells, and the epithelial layer of the pars rugosa of the endolymphatic sac. Additional studies have also confirmed the presence of peptide receptors in the inner ear.^{5,23} A study by Rachel et al²⁴ has shown that infusion of

ANP directly into the inner ear of guinea pigs participates in the regulation of vestibular blood flow. A more recent study even suggests that epithelial cells of the ELS contain an endogenous hormone, tentatively named saccin, that exerts a strong natriuresis.²⁵ In 1997, Kräuse et al²⁶ identified ANP receptors within the inner ear tissue obtained from guinea pigs by RT-PCR. They also identified a new guanylyl cyclase receptor, GC-C, which is normally present in the intestines.

The findings reported here for our current animal study represent the next step in the analysis of ANP receptors in the inner ear. The biological activity of these receptors was shown by their upregulation after injection of ANPs, supporting the hypothesis that fluid homeostasis in the endolymphatic system could be regulated by a locally effective paracrine system involving the ANP system, analogous to other organ systems in the body. The exact mechanism of action has not yet been elucidated, but intuitively would involve either regulation of fluid production by the stria vascularis or absorption by the ELS.

Although the ELS was once thought to represent a vestigial organ of embryogenesis, it is now known to have an essential role in inner ear homeostasis. The systemic administration of hypertonic agents, such as glycerol, has been shown to provoke a transient improvement in hearing in patients with the en-

dolymphatic hydrops associated with Meniere's disease, although the mechanisms for this were initially unclear.²⁷ More recent animal research has demonstrated morphological changes in the ELS associated with the systemic administration of glycerol. These changes include increased metabolic activity, with the secretion of osmotically active glycoproteins into the lumen of the sac, and subsequent lysis and breakdown of these substances.² It has thus been suggested that the ELS influences fluid homeostasis in the endolymph system through the modulation of the osmotic milieu of this space, with the secretion of osmotically active substances into its lumen; however, the mechanism of action is unknown.²⁸

It is conceivable that ANPs could be involved in this mechanism. It is likewise possible that the ANP system could be involved in fluid production by the stria vascularis.²⁴ The natriuretic properties of ANPs in the kidney are well known, and their association with excretory organs, such as the gallbladder and choroid plexus, supports this notion. One must recognize, however, the limitation of this study. While it is significant that the biological activity of ANP receptors in the inner ear has been demonstrated, their association with fluid transport in the endolymphatic system is, at this point, only conjecture. Animal studies are currently ongoing at this institution to investigate these unique peptides and define their role within the inner ear.

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FUNCTIONAL ANATOMY OF THE TENSOR VELI PALATINI MUSCLE AND OSTMANN'S FATTY TISSUE

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This study examined morphological features of the tensor veli palatini muscle (TVPM) and Ostmann's fatty tissue that may be important for eustachian tube (ET) ventilation. Histologic sections through the midcartilaginous ET from 17 human temporal bone-ET specimens (age range, 3 months to 88 years) were used to assess 1) the presence or absence of attachment of the TVPM fibers to either the perichondrium of the ET cartilage lateral lamina (LL) or a tendinous membrane along the medial margin of the TVPM, 2) the angular relationship between the TVPM fibers and the vertical axis of the ET lumen, and 3) the location of the TVPM and Ostmann's fatty tissue. The TVPM fibers were attached to the LL perichondrium in 14 cases; an attachment was absent in 3 cases because of fatty atrophy of the TVPM. However, the TVPM fibers were inserted into the tendonlike membrane in all cases. The angle of insertion of TVPM fibers into the membrane was significantly more acute (relative to the vertical ET axis) in the inferior aspect than in the superior aspect of the membrane both in young children (3 months to 4 years; mean $\pm$ SD, $39.0^\circ \pm 15.1^\circ$ superiorly to $23.8^\circ \pm 17.0^\circ$ inferiorly) and in older subjects (8 to 88 years, $30.4^\circ \pm 11.6^\circ$ superiorly to $15.7^\circ \pm 11.2^\circ$ inferiorly; *t*-test, $p < .001$). The location of Ostmann's fatty tissue accompanied the TVPM throughout the cartilaginous ET. These data suggest that contraction of the TVPM moves the LL inferolaterally to open the superior aspect more than the inferior aspect of the lumen and that Ostmann's fatty tissue will limit the opening of the ET lumen, especially that of its inferior aspect.

KEY WORDS — eustachian tube, human temporal bone, Ostmann's fatty tissue, tensor veli palatini muscle.

INTRODUCTION

The eustachian tube (ET) has 3 functions: ventilation, protection, and clearance of the middle ear.¹ Contraction of the tensor veli palatini muscle (TVPM; Fig 1), which arises from the perichondrium of the lateral lamina (LL) of the ET cartilage and the lateral membranous wall of the ET, appears to play a primary role in the opening of the ET for ventilation of the middle ear.²⁻⁷ However, the biomechanics of the actions of the TVPM during opening of the ET are poorly understood.

Ostmann's fatty tissue,⁸ located between the TVPM and the ET lumen, is thought to apply static pressure on the lateral aspect of the ET lumen when the TVPM is relaxed (Fig 1).^{6,9,10} This static pressure would tend to close the ET by compressing the lumen. However, its potential actions during active TVPM contraction have not been considered. The purpose of this study was to make an effort to understand the functional anatomy of the TVPM and Ostmann's fatty tissue for ET ventilation by histologic observation of human temporal bone-ET specimens.

MATERIALS AND METHODS

This study examined 17 human temporal bone-ET

specimens that included the ET and the ET cartilage, the TVPM, and Ostmann's fatty tissue.¹¹ They were obtained from 17 individuals without congenital anomalies or a history of ear disease. Six specimens (group A) ranged in age from 3 months to 4 years at death. Eleven specimens (group B) ranged in age from 8 to 85 years at death. These age groups were selected because the incidence of otitis media decreases markedly at 8 years of age.¹²

The specimens were fixed in 10% formalin, decalcified in 5% trichloroacetic acid, dehydrated in graded concentrations of ethanol, and embedded in celloidin. Serial 30- μ m-thick histologic vertical sections were cut in the plane perpendicular to the long axis of the ET. Every 20th section was stained with hematoxylin and eosin for light microscopic study.¹¹

The approach for studying the functional anatomy of the TVPM for ET opening is summarized in Fig 2. First, a line connecting the uppermost and lowermost points of the margin of the ET lumen at the midcartilaginous portion of the ET was defined as the vertical axis of the ET. The attachment of the TVPM fibers to the perichondrium of the LL of the ET cartilage and the tendonlike membrane of the TVPM (Fig 1) was then examined at 5 sites in the

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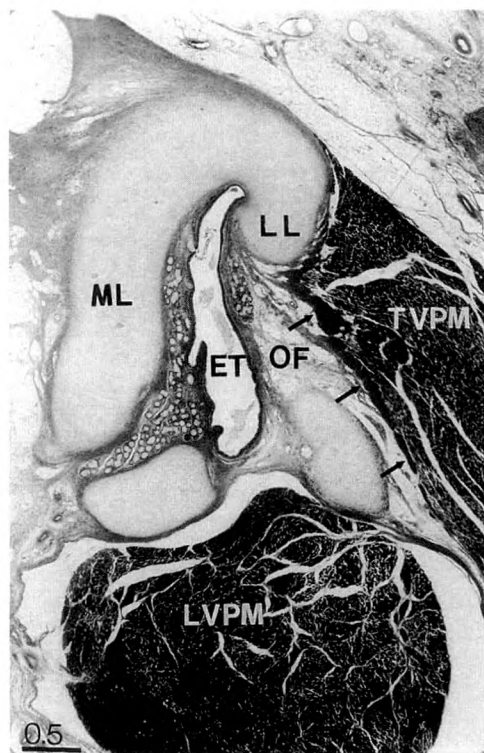


Fig 1. Photomicrograph of cross section through midcartilaginous portion of eustachian tube (ET) of 1-year-old boy. Arrows — tendinous membrane of tensor veli palatini muscle (TVPM); LL — lateral lamina of ET cartilage; LVPM — levator veli palatini muscle; ML — medial lamina of ET cartilage; OF — Ostmann's fatty tissue.

same section, designated as region x and regions a through d in Fig 2. The tendonlike membrane of the TVPM was attached to the site adjacent to the most inferior edge of the LL of the ET cartilage. We designated this site as the tip of the LL. Site x was defined as the point on the perichondrium of the LL at which the lateral surface of the LL crossed the line that was perpendicular to the vertical axis of the ET and through the midpoint between the upper margin of the ET lumen and the tip of the LL. Sites a through d were located on the tendonlike membrane of the TVPM. Site a was located at the tip of the LL. Site b was located at the point at which the tendonlike membrane of the TVPM crossed the line that was perpendicular to the vertical axis of the ET and through the midpoint of the vertical length of the ET lumen. Similarly, site d was located at the point at which the tendonlike membrane of the TVPM crossed the line that was perpendicular to the vertical axis of the ET and through the inferior tip of the ET lumen. Site c was equidistant from sites b and d. Finally, the angle between the most medial TVPM fibers and the vertical axis of the ET was measured at each of the 5 sites (x and a through d). Hypotheses regarding differences in the angles of insertion of the TVPM between sites x and a through d (within-subjects factor) and be-

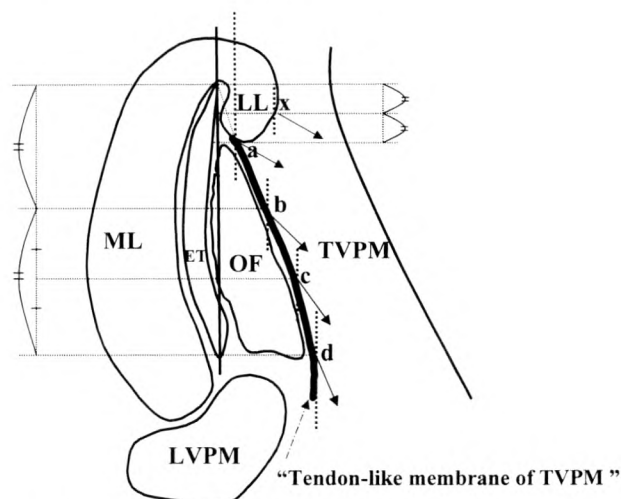


Fig 2. Schematic drawing of midcartilaginous portion of eustachian tube (ET) and its surrounding structures. Straight solid line is made connecting most upper and lower points of margin of ET. Five thick dotted lines are parallel to solid line. Arrows indicate directions of tensor veli palatini muscle (TVPM) fibers at site of 5 representative points: x, a, b, c, and d. LL — lateral lamina; LVPM — levator veli palatini muscle; ML — medial lamina; OF — Ostmann's fatty tissue.

tween age groups (between-subjects factor) were analyzed by repeated measures analysis of variance (ANOVA) and Bonferroni-corrected *t*-tests.

A different approach was used to assess the extent of the insertion of the tendonlike membrane to the LL and the extent of Ostmann's fatty tissue. First, the pharyngeal orifice of the ET was defined in each specimen as the most anterior section containing a complete cross section of the mucosal lining of the ET. This plane was equivalent to the posterior-lateral end portion of the torus tubarius. The tympanic orifice of the ET was defined as the most posterior ET cross section in proximity to the anterior-superior margin of the tympanic annulus. This plane distinguished the ET from the tympanic cavity. Second, the ET was divided into cartilaginous, junctional,¹³ and bony portions on histologic sections. Third, the insertion of the TVPM to the ET cartilage was defined as the site at which the tendonlike membrane of the TVPM attaches to the perichondrium of the tip of the LL. Similarly, the location of Ostmann's fatty tissue along the ET was defined as the site at which fatty tissue lies between the ET lumen medially and the TVPM laterally. Finally, the percentage distances from the pharyngeal orifice of the ET (relative to the length of the ET¹⁴) to both the anterior margin and the posterior margin of each tissue were calculated for each case. Unpaired *t*-tests were used to test the hypothesis that groups would differ in the relative positions of the anterior and posterior margins of the TVPM insertion and Ostmann's fatty tissue along the ET.

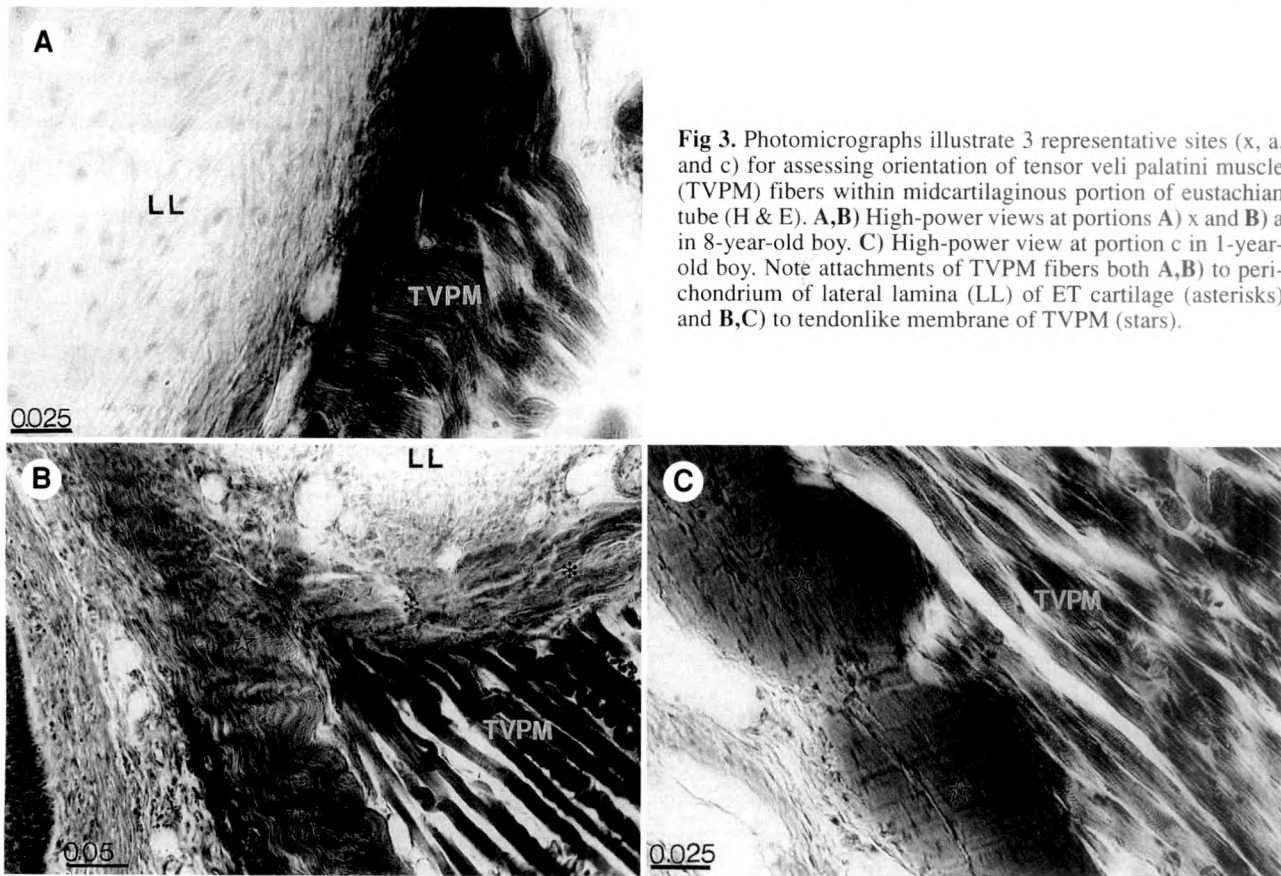


Fig 3. Photomicrographs illustrate 3 representative sites (x, a, and c) for assessing orientation of tensor veli palatini muscle (TVPM) fibers within midcartilaginous portion of eustachian tube (H & E). **A,B)** High-power views at portions **A)** x and **B)** a in 8-year-old boy. **C)** High-power view at portion c in 1-year-old boy. Note attachments of TVPM fibers both **A,B)** to perichondrium of lateral lamina (LL) of ET cartilage (asterisks) and **B,C)** to tendonlike membrane of TVPM (stars).

RESULTS

The TVPM fibers inserted directly into the perichondrium of the LL (Fig 3A) in 14 of the 17 cases. Age-related fatty atrophy of the TVPM¹⁵ disrupted the insertion into the LL in the remaining 3 cases (ages 10, 73, and 85 years). By contrast, the insertion of TVPM fibers into the tendonlike membrane was robust in all cases (Fig 3B,C).

The angles of insertion of the TVPM into the tip of the LL and into the tendonlike membrane did not differ significantly between groups. The mean values of the TVPM insertion angles at site x (attach-

ment of the TVPM fibers to the perichondrium of the LL) were $42.7^\circ \pm 14.1^\circ$ (mean $\pm$ SD; $n = 9$) in group A (Fig 4A) and $36.1^\circ \pm 10.6^\circ$ ($n = 8$) in group B (Fig 4B); these angles did not differ significantly between groups. The angles of insertion into the tendonlike membrane decreased monotonically from site a to site d in both group A and group B (repeated measures ANOVA, $F_{3,45} = 12.0$, $p < .001$). In group A (Fig 4A), the insertion angles were $39.0^\circ \pm 15.1^\circ$ at site a, $31.5^\circ \pm 12.5^\circ$ at site b, $26.0^\circ \pm 12.0^\circ$ at site c, and $23.8^\circ \pm 17.0^\circ$ at site d. For group B (Fig 4B), the angles of insertion of the TVPM fibers were $30.4^\circ \pm 11.6^\circ$ at site a, $20.9^\circ \pm 13.6^\circ$ at site b, $16.1^\circ \pm 12.3^\circ$

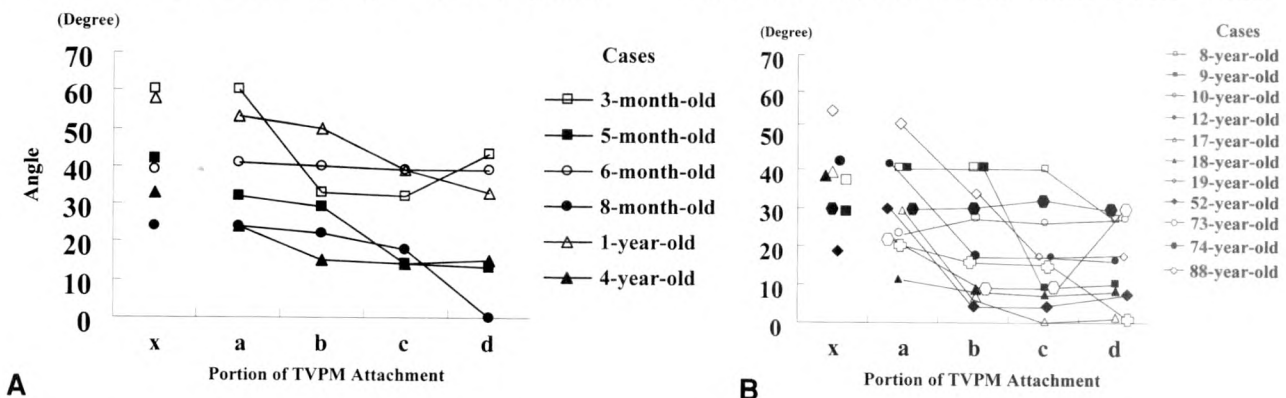


Fig 4. Orientation of tensor veli palatini muscle (TVPM) fibers with respect to eustachian tube lumen at 5 representative sites (x, a, b, c, and d). **A)** Group A (younger). **B)** Group B (older).

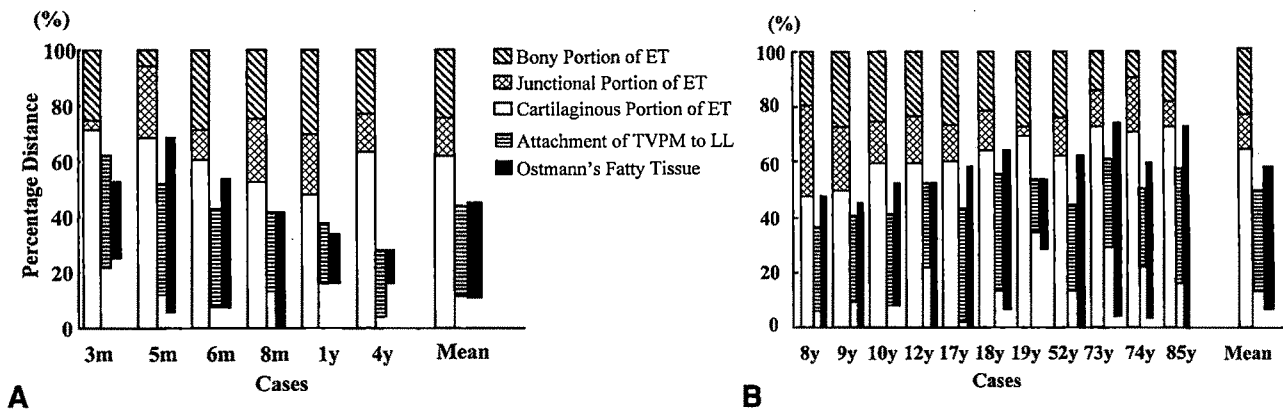


Fig 5. Spatial relationships between eustachian tube (ET), attachment of tensor veli palatini muscle (TVPM) fibers to lateral lamina (LL) of ET cartilage, and Ostmann's fatty tissue. **A)** Group A (younger). **B)** Group B (older).

at site c, and $15.7^\circ \pm 11.2^\circ$ at site d. However, there were no significant differences between the angles of insertion in the two groups (repeated measures ANOVA; main effect of group; $F_{1,15} = 2.8$; $p = .1131$) or the angles of insertion at x and a in either group A or group B (t -test, $p > .5$).

The mean locations of the anterior margin and posterior margin of the TVPM were $12.4\% \pm 6.3\%$ and $44.0\% \pm 11.9\%$ of the length of the ET (from the pharyngeal orifice) in group A (Fig 5A) and $15.9\% \pm 10.2\%$ and $48.7\% \pm 8.1\%$ of the length of the ET in group B (Fig 5B). These locations did not differ significantly between groups. The mean locations of the anterior margin and posterior margin of Ostmann's fatty tissue were $11.6\% \pm 9.0\%$ and $46.3\% \pm 14.9\%$ in group A (Fig 5A) and $4.6\% \pm 8.4\%$ and $58.4\% \pm 9.5\%$ in group B (Fig 5B), respectively. In group A (children 3 months to 4 years old), the borders of the TVPM and Ostmann's fatty tissue were aligned; ie, neither the locations of the anterior margins of the TVPM and Ostmann's fatty tissue (t -test; $t(10) = 0.182$, $p = .859$) nor the locations of the posterior margins of the two structures (t -test, $t(10) = 0.301$; $p = .767$) differed significantly. However, in group B (specimens greater than 8 years old), the borders of Ostmann's fatty tissue extended beyond the borders of the TVPM. The anterior margin of Ostmann's fatty tissue extended anteriorly to the corresponding border of the TVPM (t -test; $t(20) = 2.860$; $p = .0097$), and its posterior margin extended posteriorly to the posterior border of the TVPM (t -test; $t(20) = 2.597$; $p = .0175$).

DISCUSSION

Contraction of the TVPM has been thought to open the ET lumen by actions at 2 sites: 1) insertion of fibers into the perichondrium of the LL of the ET cartilage^{3,6} and 2) insertion of fibers into the lateral membranous wall of the ET.⁷ The latter fibers were termed *dilatator tubae* by Rood and Doyle,¹⁶ reflect-

ing the assumption that they dilate the lumen by an inferolateral displacement of the membrane. However, this view could be discussed with the findings reported by Matsune et al³ that the lateral membranous wall of the ET inserts into the perichondrium of the tip of the LL of the ET cartilage. This finding implies that the net action of the TVPM is directed at the tip of the LL, via both direct actions and traction on the LL via the tendinous lateral wall of the ET. Because both of these actions pull the tip of the LL inferolaterally, the lateral membranous wall of the ET is more appropriately termed a tendinous membrane of the TVPM. Further, Sando et al⁶ hypothesized that this inferolateral movement of the LL, due to the contraction of the TVPM, is an efficient mechanism for active ET opening and that the ET ventilation is maintained mainly in the upper (superior) half of the ET lumen. However, we should also be aware that the action of the TVPM may produce a certain lateral movement of the membrane, causing direct dilation of the lateral wall of the ET lumen.

Previous studies have reported that the TVPM is oriented at an angle of approximately 10° relative to the ET lumen and at an angle of approximately 80° relative to the LL of the ET cartilage.^{17,18} However, it appeared from the histologic sections that the angle of insertion of the TVPM fibers varied between the tip of the LL and the inferior margin of the insertion into the lateral wall of the ET. Thus, the present study measured the angle between the TVPM fibers and the ET lumen at 5 standard sites relative to the vertical axis of the ET, ranging from the lateral tip of the LL to the level of the inferior margin of the ET lumen.

One main finding of this study is that the angle between the TVPM and the vertical axis of the ET lumen decreases in a superoinferior direction. This angle of action of the TVPM deviates approximately 40° from vertical at the level of the LL tip, but it

deviates only 15° to 20° from vertical at the level of the inferior margin of the ET lumen. This finding suggests that contraction of the TVPM will pull the LL inferolaterally, dilating the superior aspect of the ET lumen more than the inferior aspect. Two factors appear to contribute to this action: 1) TVPM fibers that insert directly into the LL and the junction between the tendinous membrane and the LL tip (site a) and 2) traction on the tip of the LL by the tendinous membrane. These considerations suggest that one main action of the TVPM is dilation of the superior aspect of the ET lumen more than the inferior aspect. It will be of interest to determine the degree of impairment of ET ventilatory function in patients

with age-related fatty atrophy of the TVPM fibers that insert directly into the LL.

Ostmann's fatty tissue⁸ forms a pad between the tendinous membrane and the ET lumen. It is roughly triangular in cross section, with its base located inferiorly and its apex superiorly. Hence, when the tendinous membrane is shifted inferolaterally by contraction of the TVPM, Ostmann's fatty tissue is expected to limit the opening of the ET, especially its inferior aspect. Thus, these findings are consistent with the hypothesis that contraction of the TVPM facilitates ventilatory function mainly in the superior aspect of the ET lumen.

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INTRAMUSCULAR HEMANGIOMA OF THE POSTERIOR BELLY OF THE DIGASTRIC MUSCLE FAILING TO HIGHLIGHT ON MAGNETIC RESONANCE IMAGING

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Intramuscular hemangiomas of the digastric muscle are rare. We present the fifth case reported and the second to be imaged in vivo with magnetic resonance imaging. Accurate preoperative diagnosis of these lesions is difficult, but may be facilitated by a high degree of clinical suspicion and imaging. Magnetic resonance imaging using T2-weighted sequencing has been reported to effectively highlight these lesions. We present a case in which magnetic resonance imaging failed to highlight an intramuscular hemangioma of the posterior belly of the digastric muscle. Possible reasons for this failure are discussed.

KEY WORDS — digastric muscle, hemangioma, magnetic resonance imaging.

INTRODUCTION

Intramuscular hemangiomas are rare, constituting 0.8% of all hemangiomas.¹ Between 14% and 21% of all intramuscular hemangiomas occur in the head and neck,² in which diagnosis is often difficult even with accurate preoperative imaging. Hemangiomas of the digastric muscle can be difficult to distinguish clinically from salivary gland neoplasms³⁻⁶; those of the posterior belly are frequently mistaken for parotid lesions, and those of the anterior belly for submandibular gland lesions. Magnetic resonance imaging (MRI) has been recommended by several authors⁶⁻⁹ as the investigation of choice in hemangiomas, as these lesions give a high-intensity signal on T2-weighted sequencing.

CASE REPORT

A 31-year-old woman presented with a 2-month history of a gradually enlarging mass in the region of her right parotid gland. The mass was associated with a dull ache centered at the angle of her jaw. The ache occurred approximately twice a week and would last for up to 2 hours. It would radiate along the line of the sternocleidomastoid muscle. Precipitating factors included exercise, warm weather, and stress. She denied any trauma to her neck, but in her job as a personal assistant, she frequently held a telephone to her right ear with her shoulder.

She had noted a lump in this area 3 years earlier

and attended another hospital. The physician was unable to palpate any lesion in the parotid region, but suspected a mass lower in the neck. Surgical exploration of the mass identified no lesion. The patient thought that the initial lump had disappeared spontaneously.

On our examination, a fullness was palpable in the tail of the right parotid gland. The right sternocleidomastoid muscle was hypertrophied, and there was a transverse scar in the lower part of the neck, crossing both the sternal and clavicular heads of this muscle. No other abnormalities were detected. Fine-needle aspiration yielded only parotid tissue.

In view of the clinical picture and on discussion with the patient, a "wait and watch" policy was adopted. She was reviewed 6 months later, and a further fine-needle aspiration was performed. This again demonstrated only parotid tissue. An MRI scan (Fig 1) was then performed, and the report commented that the lesion was thought to arise within the deep lobe of the parotid gland, but the lack of demarcation from the posterior belly of the digastric muscle made this location uncertain. On review with these results, the patient elected for surgical excision.

Excision was performed under general anesthesia by a standard parotidectomy incision. A superficial parotidectomy was performed, allowing identification of the mass in the posterior belly of the digastric

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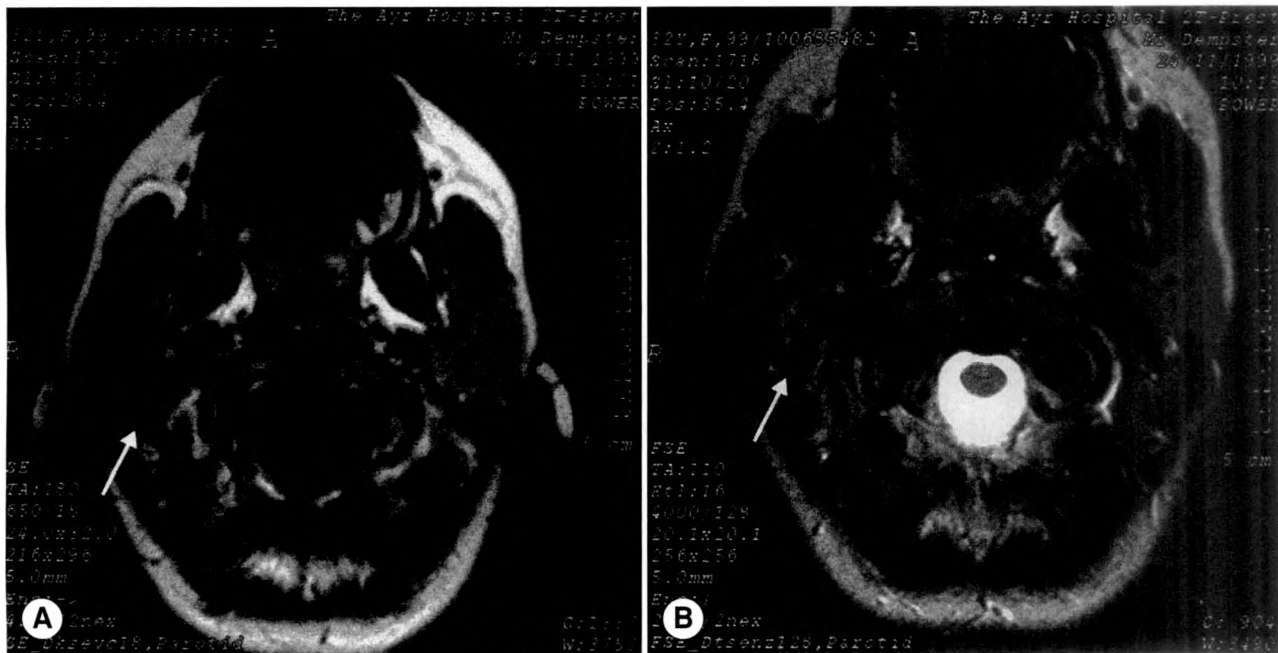


Fig 1. Magnetic resonance axial sections at level of parotid gland demonstrate mass (arrows) in right digastric muscle. **A)** T1-weighted. Lesion gives low-intensity signal isointense to muscle. **B)** T2-weighted. Signal intensity of mass is now similar to that of parotid soft tissue. Notice areas of signal void within mass due to blood vessels and hemosiderin.

muscle. The mass was then excised. Histology revealed a mixed-type hemangioma (Fig 2).

After the operation, the patient progressed well, but did complain of some neuralgic type of pain in the area of the wound, which was treated with amitriptyline hydrochloride. On review at 8 months, some fibrosis in the scar was palpable, but there was no evidence of recurrence.

DISCUSSION

Hemangiomas of the head and neck are rare, and

intramuscular hemangiomas make up a small percentage of these. The cause of these lesions is unknown and is presumed to be congenital, but trauma and hormonal influences have been suggested.¹⁰ Only 4 other cases of hemangiomas of the digastric muscle have been reported in the world literature.³⁻⁶ The relevant details of these cases, as well as those of our patient, are summarized in the Table.³⁻⁶ Intramuscular hemangiomas arising from the posterior belly of the digastric muscle have only been reported in 2 other cases.^{3,6} In both, an intraparotid lesion was suspected before operation, but the actual site of the neoplasm

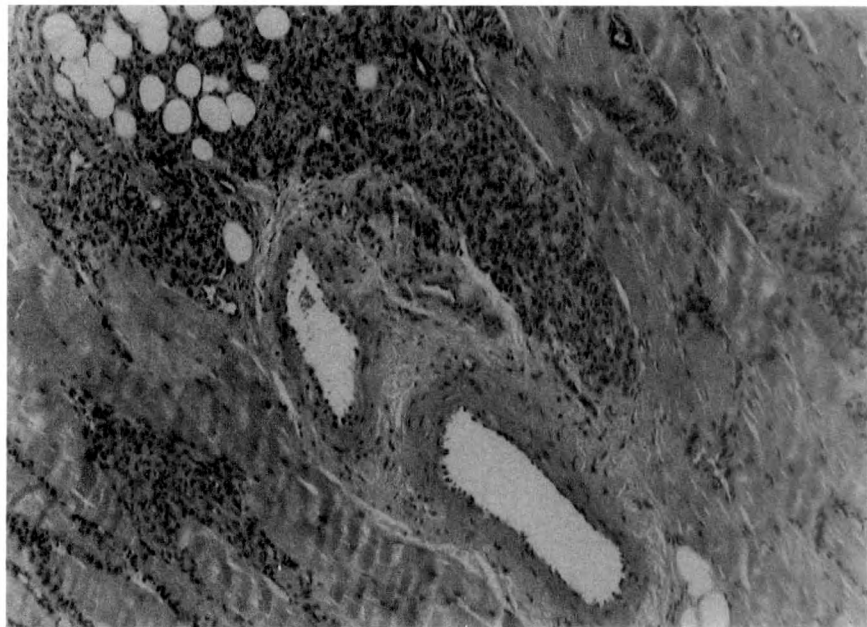


Fig 2. Mixed vessel hemangioma demonstrates both capillaries and large vessels (H & E, original $\times 100$).

CLINICAL PRESENTATION, SITE, HISTOPATHOLOGY, INVESTIGATIONS, AND TREATMENT IN FIVE REPORTED CASES OF INTRAMUSCULAR HEMANGIOMA OF DIGASTRIC MUSCLE

<i>Authors</i>	<i>Date</i>	<i>Age at Presentation (y)</i>	<i>Sex</i>	<i>Duration of Symptoms (mo)</i>	<i>Pain</i>	<i>Site (Belly of Digastric Muscle)</i>	<i>Histopathologic Classification</i>	<i>Investigations</i>	<i>Correct Preoperative Diagnosis</i>	<i>Treatment</i>
Clemis et al ³	1975	42	F	12	No	Posterior	Mixed	Not stated	No	Surgical excision
Slack et al ⁴	1989	16	F	5	No	Anterior	Capillary	FNA, radiography, sialography	No	Surgical excision
Sayan et al ⁵	1992	57	F	3	No	Anterior	Cavernous	Radiography, sialography, CT, US	Yes	Surgical excision
Sichel et al ⁶	1998	42	M	3	No	Posterior	Not stated	FNA, CT, MRI	No	Surgical excision
This study	2001	31	F	2	Yes	Posterior	Mixed	MRI	No	Surgical excision

FNA — fine-needle aspiration, CT — computed tomography, US — ultrasonography, MRI — magnetic resonance imaging.

was not identified until the time of surgery.

One was diagnosed during operation with the aid of frozen section, and the other was diagnosed by histopathology after the operation. Only 1 of these hemangiomas was submitted to MRI before operation.⁶ The authors of the report commented that in retrospect, they believed that more careful interpretation of the MRI findings could have led to a more precise preoperative diagnosis, because hemangiomas are the only described tumor of the digastric muscle, and on review of the MRI findings, the mass could be seen arising distinctly from the posterior belly of the digastric muscle. They also commented that the lesion gave a high-intensity signal on T2-weighted sequencing MRI, and this finding is consistent with the diagnosis of a hemangioma.

Several authors^{7,8,11} have shown that intramuscular hemangiomas demonstrate low to intermediate signal intensity on T1-weighted MRI but are bright, having a high signal intensity, on T2-weighted sequencing. On histology, hemangiomas contain variable amounts of nonvascular tissue such as fat, smooth muscle, fibrous tissue, myxoid stroma, hemosiderin, thrombus, and even, rarely, bone,¹² as well as vascular elements. Phleboliths can also occur and can aid in the radiologic diagnosis. Certain radiologic features on MRI are suggestive of hemangiomas: 1) high-intensity (bright) signal on T2-weighted imaging; 2) striated-septated (serpentine) configuration correlating with fibro-fatty septa between endothelial-lined vascular channels in lesions greater than 2 cm; and 3) focal heterogeneous areas of thrombus, fibrosis, hemosiderin deposition, and/or calcification.^{7,8,11}

Buetow et al⁷ commented that the characteristic MRI appearances of hemangiomas are due to the combination of large vessels, containing stagnant blood, and the nonvascular elements, which consist

predominantly of fatty and fibrous tissues. If the brightness on T2-weighted imaging were due mainly to the volume of stagnant blood, then small vessel or capillary hemangiomas should not give a high-intensity signal. However, small vessel hemangiomas give a high-intensity signal on T2-weighted MRI.^{2,9} It is possible, in our case, that compression by surrounding tissues may have prevented stagnation of blood in the vessels and was therefore the reason for the failure of MRI to highlight our patient's hemangioma. Sichel et al,⁶ however, in reporting the only other case of an intramuscular hemangioma of the posterior belly of the digastric muscle to have been submitted to MRI, said that T2-weighted MRI gave a high signal intensity. Therefore, we postulate that the most likely reason, in our case, for the failure of T2-weighted MRI to show a high-intensity signal was the composition of the nonvascular elements.

The management of intramuscular hemangiomas should be individualized, depending on tumor location, accessibility, depth of invasion, patient age, and cosmetic considerations.^{10,13} Surgical excision has long been recommended as the treatment of choice,^{2,10,12} but more recently, this has been questioned, and some surgeons now recommend "watchful waiting."¹³ It should be noted that these lesions can be difficult to distinguish, both clinically and radiologically, from malignant soft tissue tumors, especially angiosarcomas.^{2,12,14} Fine-needle aspiration adds no information of any clinical value in the diagnosis of these vascular lesions, but should be performed to aid in the exclusion of other soft tissue tumors. Other treatment modalities have been used to treat these lesions with limited success, and these include radiotherapy, sclerosing agents, and corticosteroids.^{10,12,13} Preoperative embolization has been reported as the sole treatment modality in selected cases and may be of use as an adjunct to surgery.^{10,13}

In terms of histology, these are benign neoplasms. They do not appear to regress spontaneously, unlike pediatric cutaneous and parotid hemangiomas.^{2,9} They frequently recur if incompletely excised, with recurrence rates varying within the pathological subgroups.¹² Recurrence rates of 20%, 9%, and 28% have been reported for capillary, large vessel, and mixed-type intramuscular hemangiomas, respectively.¹² Two of the 3 reported cases of intramuscular hemangiomas of the posterior belly of the digastric muscle underwent superficial parotidectomy before the mass was identified as arising from this site. Accurate preoperative diagnosis could have led to better preoperative planning, and the procedure could have been carried out without the need for superficial parotidectomy and the morbidity associated with this

procedure.

In conclusion, MRI is useful in the diagnostic workup of head and neck soft tissue tumors. In any soft tissue mass seen to be arising from the digastric muscle, the most likely diagnosis is that of hemangioma. Accurate interpretation of MRI in this region remains difficult because of the rarity of this tumor. It should be noted that not all intramuscular hemangiomas will give a high-intensity signal on T2-weighted MRI. Magnetic resonance imaging is a very useful tool in identifying whether a mass is a soft tissue tumor, but is not always helpful in differentiating between histopathologic types. Surgical excision remains the treatment of choice for head and neck intramuscular hemangiomas.

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POSTOPERATIVE OLFACTION IN CHRONIC SINUSITIS: SMOKERS VERSUS NONSMOKERS

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In order to study the efficacy of surgical intervention, as well as the influence of cigarette smoking on olfaction in clinical states, we examined olfactory ability by means of the University of Pennsylvania Smell Identification Test (UPSIT). We enrolled 37 patients 21 to 60 years of age who underwent surgery for chronic sinusitis. Normosmia was defined as an UPSIT score of 30 or more. Among these patients, 31 had a disturbance in their smelling ability in the preoperative stage, and the improvement rate, defined as a rise in score of 4 or more points after surgery, was 64.5%; 11 were well healed. However, all 7 patients who were more than 40 years old at the time of surgery, and currently smoking cigarettes, had a significant deficit in olfaction in the preoperative stage and even in the postoperative stage. This study demonstrated that cigarette smoking has adverse effects on olfactory ability, especially for older patients.

KEY WORDS — chronic sinusitis, functional endoscopic sinus surgery, olfactory function, smoking, University of Pennsylvania Smell Identification Test, UPSIT.

INTRODUCTION

Olfaction is a major sensory system; in humans, it serves as an early warning system for the detection of dangerous fumes or spoiled food, as well as a means of sensing food flavors. The accurate assessment of olfactory function is important for detecting chemosensory problems related to aging, environmental pollution, and various diseases.

Clinically, olfactory disturbance mostly results from chronic rhinosinusitis, and endoscopic sinus surgery has been reported to yield a beneficial outcome for this dysfunction.¹⁻³ In typical practice, however, the evaluation is based only on each patient's self-reported symptoms, and rarely is olfaction tested quantitatively or used as a criterion for improvement. Cigarette smoking is also known to be a risk factor for the development of olfactory dysfunction. Smoking-induced olfactory dysfunction has been described in several reports, but exclusively in volunteers,^{4,5} and clinical evidence has not yet been well documented.

The purpose of this study was to focus on the benefits of functional endoscopic sinus surgery and to detect the influence of cigarette smoking on olfactory function in patients with rhinosinusitis, on the basis of an objective criterion: the University of Pennsylvania Smell Identification Test (UPSIT). This test incorporates 40 microencapsulated odors, and has been shown to be highly reliable and to correlate well with detection threshold tests.⁶ The test is also sen-

sitive to various smell deficits and is widely used in Europe and North America. Kondo et al⁷ showed that it was applicable to the Japanese population. We used the UPSIT before and after surgical intervention to evaluate smelling ability, as well as to evaluate differences between smokers and nonsmokers.

SUBJECTS AND METHODS

We studied 37 patients (30 men and 7 women; age range, 21 to 60 years; mean $\pm$ SD, 42.8 $\pm$ 11.9 years) who underwent functional endoscopic sinus surgery from 1997 to 1999 at Meijo Hospital, Nagoya, Japan. The subjects had had chronic rhinosinusitis for 3 months or longer that failed to be improved by pharmacological intervention. Of these subjects, 13 (35.1%) were cigarette smokers, and 18 (48.6%) had undergone previous surgical intervention for their nasal disease.

Functional endoscopic sinus surgery was performed as described by Kennedy et al⁸ under local anesthesia with 10% cocaine hydrochloride and 0.5% lidocaine hydrochloride in 1:100,000 of epinephrine. Additional sedation was obtained by use of intravenous propofol.^{9,10} In some cases, a KTP laser was used to cut polyps. Twelve operations (32.4%) were performed in combination with inferior turbinectomy and/or septal surgery. The follow-up period ranged from 3 to 24 months.

Before and after operation, a detailed computed tomography scan was performed transversely and co-

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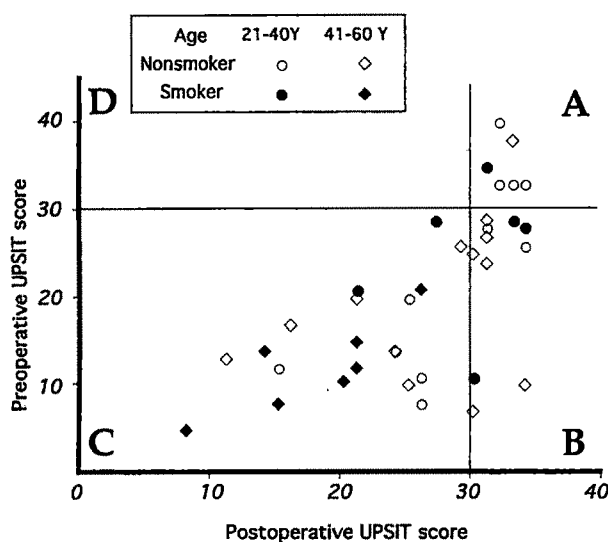


Fig 1. University of Pennsylvania Smell Identification Test (UPSIT) scores of all 37 subjects in preoperative and postoperative stages. A — both preoperative and postoperative UPSIT scores of ≥ 30 (normosmia group); B — preoperative UPSIT score of < 30 and postoperative UPSIT score of ≥ 30 (well-healed group); C — both preoperative and postoperative UPSIT scores of < 30 (poor-prognosis group); D — preoperative UPSIT score of ≥ 30 and postoperative UPSIT score of < 30 (worse group).

ronally with 2-mm interleaved scans to evaluate the extent of disease. Each paranasal sinus (frontal, maxillary, anterior ethmoid, posterior ethmoid, and sphenoid) was assessed on the basis of the degree of obliteration (none on one side, 0 points; cloudiness, 1 point; partial obliteration, 2 points; complete obliteration; 3 points). The points for both sides were added, and each sinus was scored as follows: score of 0 = 0 or 1 point; score of 1 = 2 or 3 points; score of 2 = 4 to 6 points. The scores were added together, for a maximum possible score of 10. Opacification in the region of the olfactory cleft was also calculated as follows: score of 0 = both sides open; score of 1 = one side open; and score of 2 = both sides opaque.

Olfactory function was assessed with the UPSIT. All patients completed the test in both the preoperative and postoperative stages. Because of cultural factors, the UPSIT scores of Japanese subjects are typically 5 points lower than those of American subjects of equivalent age.⁷ In the present study, normosmia was defined as an UPSIT score of 30 or more. On the basis of this definition, the 37 patients were classified into 4 groups: normosmia or group A (preoperative and postoperative UPSIT scores of ≥ 30); well-healed or group B (preoperative UPSIT score of < 30 and postoperative UPSIT score of ≥ 30); poor-prognosis or group C (preoperative and postoperative UPSIT scores of < 30); and worse or group D (preoperative UPSIT score of ≥ 30 and postoperative UPSIT score of < 30).

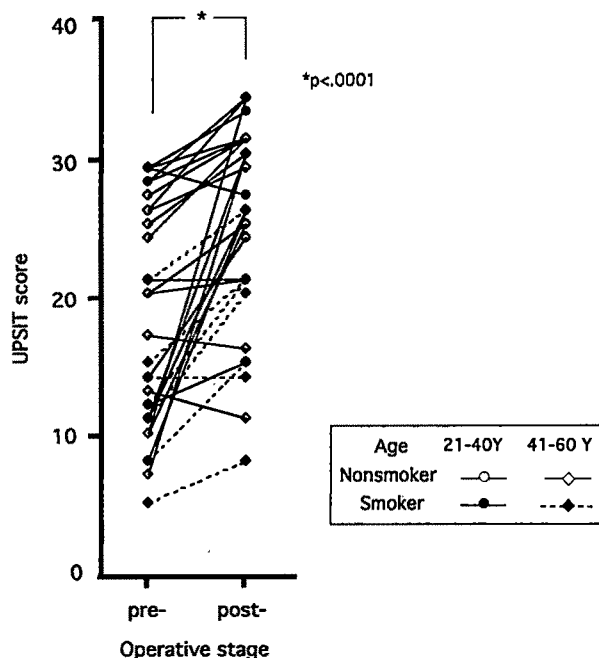


Fig 2. Efficacy of surgical intervention for olfactory function in 31 patients with hyposmia defined as UPSIT score of < 30 .

To assess the efficacy of the surgical procedure for olfaction, we examined the difference in UPSIT scores between the preoperative and postoperative stages for 31 subjects who had hyposmia before surgery. In addition, we statistically compared various possible prognostic factors, such as radiographic findings, age, gender, cigarette smoking, duration of disease, and laser use, between the well-healed group B and the poor-prognosis group C. As for age, the patients were divided into 2 groups: 12 patients in a younger group (21 to 40 years of age) and 19 in an older group (41 to 60 years of age). The correlation of postoperative UPSIT scores with a smoker's age and with the smoking dose was studied. The smoking dose was calculated in pack-years by multiplying the number of packs smoked per day by the number of years of smoking.

Fisher's exact test and the Mann-Whitney test were used to compare the groups. The Wilcoxon signed rank test was used to compare UPSIT scores between the preoperative and postoperative stages in those who had hyposmia before surgery; a *p* value of less than .05 was considered significant. The Spearman correlation coefficient between the measures was also computed.

RESULTS

As shown in the scatter diagram in Fig 1, of 37 patients, 6 patients (group A) continued to be classified as normosmic and 11 (group B) were well healed after surgery, whereas 20 (group C) remained hyposmic in spite of the operative procedure. None were

TABLE 1. COMPARISONS OF RADIOGRAPHIC FINDINGS BETWEEN WELL-HEALED GROUP B AND POOR-PROGNOSIS GROUP C

Group	Well-Healed Group B (n = 11)		Poor-Prognosis Group C (n = 20)		Mann- Whitney Test p
	25th-75th		25th-75th		
	Median	Percentile	Median	Percentile	
CT score*					
Preoperative	4.0	2.3-7.8	4.5	3.0-8.5	.506
Postoperative	1.0	0.0-3.0	1.5	0.0-4.0	.756
Cleft score*					
Preoperative	1.0	1.0-2.0	2.0	0.0-2.0	.946
Postoperative	0.0	0.0-0.0	0.0	0.0-0.0	.914
CT — computed tomography.					
*See text for scoring method.					

CT — computed tomography.

*See text for scoring method.

worse (group D). To assess the efficacy of the surgical procedure for olfaction, we compared the UPSIT scores between the preoperative and postoperative stages for the 31 subjects in groups B and C.

A change of 4 or more points in the UPSIT score after surgery is regarded as meaningful.¹¹ Of 31 patients regarded as hyposmic before operation, 20 patients (64.5%) presented meaningful improvement (Fig 2; $p < .001$; Wilcoxon's signed rank test). Some patients, however, were still hyposmic after the operation.

As for the radiographic findings, no significant difference was found between well-healed group B and poor-prognosis group C (Table 1; Mann-Whitney test). Potential prognostic factors that affect olfaction, such as age, sex, cigarette smoking, duration of disease, and use of laser, were examined statistically (Table 2).

TABLE 2. COMPARISONS OF VARIOUS PROGNOSTIC FACTORS BETWEEN WELL-HEALED GROUP B AND POOR-PROGNOSIS GROUP C

Factor	Well-Healed Group B (n = 11)		Poor-Prognosis Group C (n = 20)		Fisher's p
	No.	%	No.	%	
Age					
21-40 y (n = 12)	5	42	7	58	.705
41-60 y (n = 19)	6	32	13	68	
Sex					
Female (n = 6)	3	50	3	50	.638
Male (n = 25)	8	32	17	68	
Cigarettes					
Nonsmoker (n = 19)	8	42	11	58	.452
Smoker (n = 12)	3	25	9	75	
Duration of disease					
≤10 y (n = 20)	10	50	10	50	.047
>10 y (n = 11)	1	9	10	91	
Laser use					
With (n = 18)	4	22	14	78	.128
Without (n = 13)	7	54	6	46	

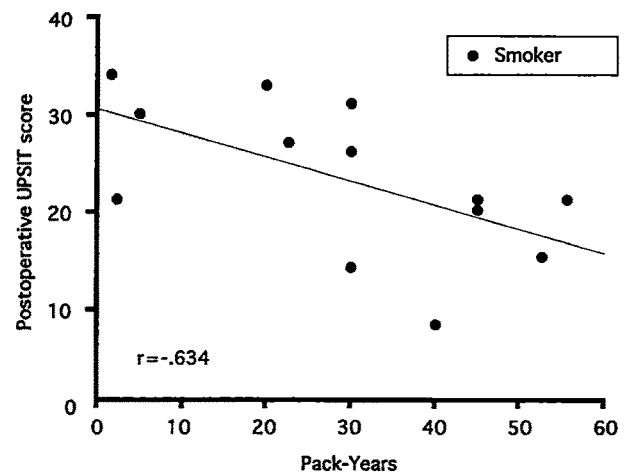


Fig 3. Relationship between cumulative smoking dose (see text) and postoperative UPSIT score in 13 smokers.

When merely comparing the treatment outcomes between nonsmokers and smokers, we did not find sufficient evidence to conclude a difference between them (Table 2). The power of the test to detect a difference of at least 20 percentage points between smokers and nonsmokers (poor prognosis of 75% versus 58%) was only 0.17. When analyzing the relationship between the cumulative dose of cigarette smoking and the postoperative UPSIT score, we found a significant negative correlation (Fig 3; $r = -.634$; $p = .005$; Spearman correlation). Figure 2 shows that although older smokers (closed diamonds) definitely improved after operative intervention, none of them achieved UPSIT scores of 30: they all remained in poor-prognosis group C (Fig 1). Furthermore, analyzing the correlation between the postoperative UPSIT score and age revealed a strongly negative relationship in the smoking group (Fig 4B; $r = -.802$; $p = .008$; Spearman correlation), which was not seen in the nonsmoking group (Fig 4A).

The duration of the disease also had significant effects (Table 2); subjects who had symptoms for more than 10 years had a worse outcome. No significant difference between the genders was observed, and the KTP laser procedure had no marked influence. Although allergic rhinitis appeared not to affect the olfactory prognosis, patients with asthma had a much worse objective outcome (data not shown).

DISCUSSION

Patients with sinusitis and polyposis often have troublesome olfactory disturbances related to induced nasal obstruction. Other mechanisms, such as changes in the composition and function of the olfactory mucus due to hypersecretion and hyposcretion, and dysfunction of the olfactory receptor cells caused by toxic inflammatory mediators, must be taken into consideration.¹²

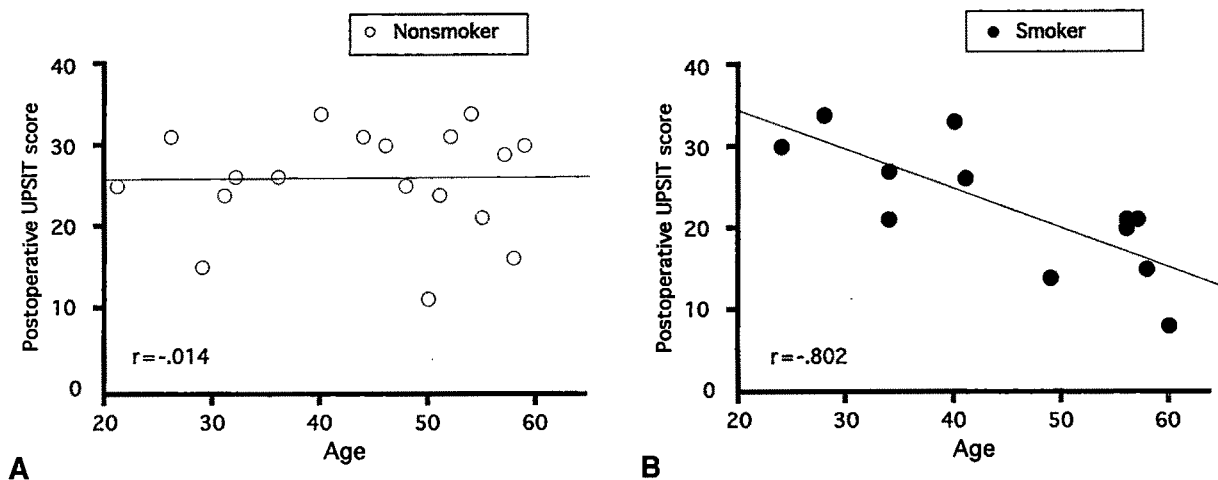


Fig 4. Relationship between age and postoperative UPSIT score for A) nonsmokers and B) smokers.

Endoscopic sinus surgery has been reported to be beneficial in mitigating some, but not all, types of olfactory dysfunction. In this study, we evaluated olfactory ability as measured by the UPSIT and found that the postoperative values significantly improved at a rate of approximately two thirds (Fig 2). Sometimes, however, it was difficult to achieve successful improvement in olfaction, in spite of appropriate surgical intervention. Although the opacification in the region of the cribriform plate disappeared after operation (Table 1), some subjects gained no practical improvement in the ability to smell — a finding indicating the complexity of olfactory dysfunction.

In volunteers who had no apparent sinonasal tract disease, the adverse effects of cigarette smoking on olfactory function have been documented.^{4,5} In 1990, Frye et al⁴ described an inverse association between cumulative smoking dose and UPSIT scores. We obtained very similar results in this study, as shown in Fig 3. According to the postoperative UPSIT scores, nonsmokers lost little olfactory ability throughout the 21- to 60-year age range examined, whereas smokers were found to develop olfactory dysfunction that was highly related to aging. These disabilities remained even after surgical intervention for smokers more than 40 years old (Fig 4B). Doty et al¹³ demonstrated age-related decreases in olfactory function. The present data indicate that it is important to consider the additional interaction between aging and cigarette smoking when evaluating olfaction. It may be that cigarette smoking causes time-related alterations in the olfactory system via its long-standing intranasal neurotoxicity, inducing dysfunction of ol-

factory receptor cells and subsequently reducing the ability to smell for older patients. To confirm this possibility, further study will be required.

Allergic rhinitis appeared to have little or no significant influence. However, patients with asthma have a much worse objective outcome; it is difficult to maintain adequate smelling ability after surgery.¹⁴ Some patients who have undergone prior rhinotomy had adhesion formation around the cribriform plate and cannot make a good recovery; this finding suggests that the surgical procedure might injure the olfactory mucosa and lead to an impaired sense of smell.¹⁵ In our subjects, some adhesion formations were noted after operation within the middle meatus; the resulting complications were considered to be one of the causes of impaired olfaction.

In conclusion, this study demonstrates that endoscopic sinus surgery is effective in reversing olfactory disturbance and that the UPSIT is highly valuable not only for assessing the efficacy of surgical intervention on olfaction, but also for analyzing the mechanism of olfactory disturbance. This study also highlights the fact that cigarette smoking is one of the most damaging influences on the smell function. In Japan, the rate of smoking is twice as high as that in Europe and North America. We have illustrated the toxic effect of cigarette smoking to be important in clinical practice, and further studies are needed to elucidate the subtle effects of smoking on patients with chronic sinusitis and nasal polyposis. Thus, whether the patient is a smoker has an influence on postoperative outcomes.

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**ANALYSIS OF SPIRAL GANGLION CELL
POPULATIONS IN CHILDREN WITH NORMAL
AND PATHOLOGICAL EARS**

**TREATMENT OPTIONS FOR LYMPHANGIOMA
IN INFANTS AND CHILDREN**

**REPAIR OF A TYPE IV
LARYNGOTRACHEOESOPHAGEAL CLEFT
WITH CARDIOPULMONARY BYPASS**

**PARAGANGLIOMA OF THE CERVICAL
SYMPATHETIC TRUNK**

**HEARING LOSS IN USHER SYNDROME TYPE II
IS NONPROGRESSIVE**

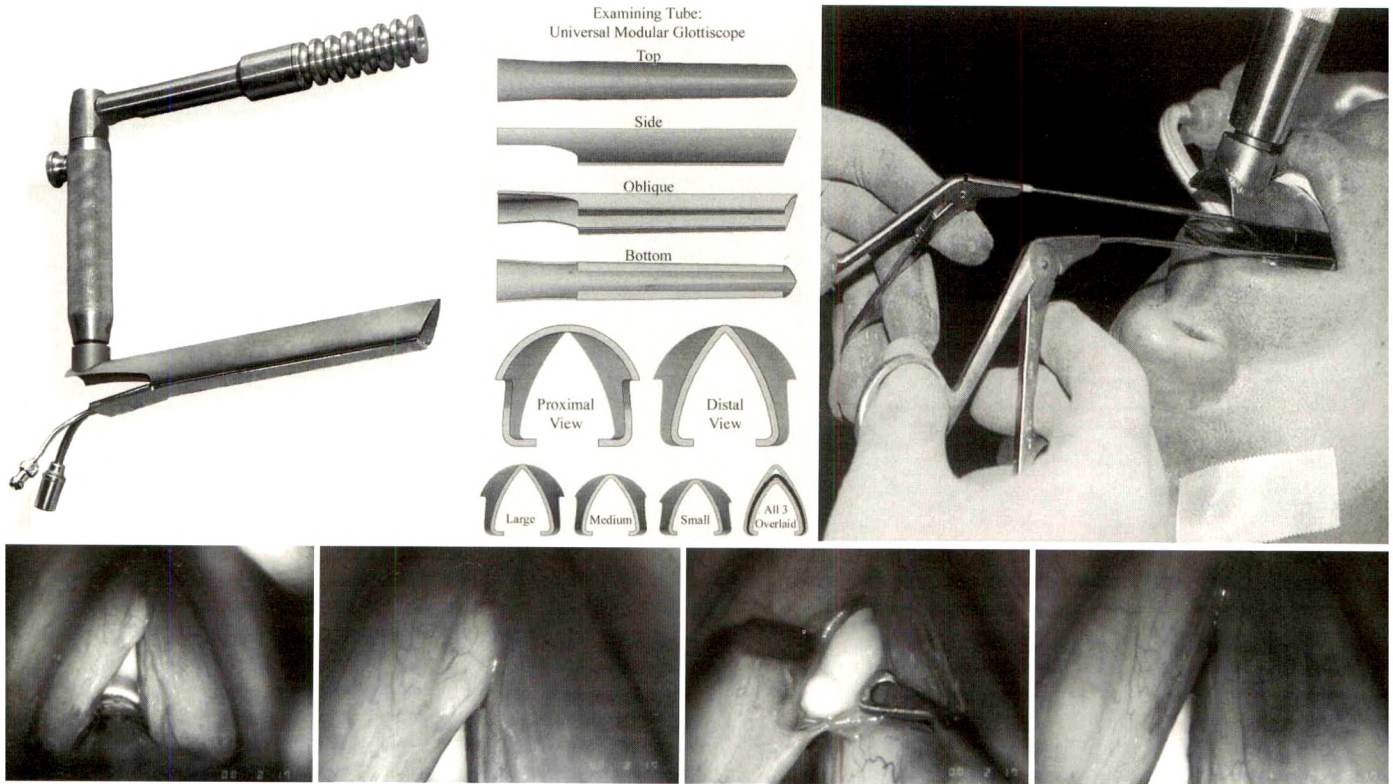


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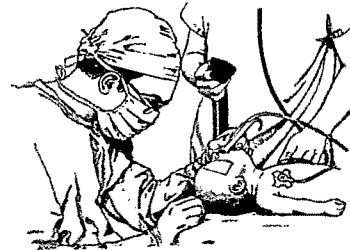
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ANALYSIS OF SPIRAL GANGLION CELL POPULATIONS IN CHILDREN WITH NORMAL AND PATHOLOGICAL EARS

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This study analyzed features of total and segmental spiral ganglion cell populations in children with normal ears and those with various pathological conditions. Sixty-three human temporal bone specimens, obtained from 43 children 4 days to 9 years of age, were studied histopathologically. These specimens were divided into 5 diagnostic groups: group 1, normal ears (13 ears); group 2, congenital infectious diseases (13 ears); group 3, chromosomal aberrations (11 ears); group 4, multiple craniofacial anomalies with hereditary or genetic causes (21 ears); and group 5, perinatal and postnatal asphyxia (5 ears). Eighteen of the 63 ears had documented profound deafness. In either normal ears (group 1) or those with various pathological conditions (groups 2 through 5), the total number of ganglion cells did not change as a function of age during the first 10 years. The total number of ganglion cells was significantly larger in group 1 (33,702) than in each of groups 2, 3, 4, and 5 ($p < .01$), and the number was significantly larger in group 2 than in each of groups 4 and 5 ($p < .01$ and $p < .05$, respectively). The ratio of basal to apical ganglion cell populations remained constant in both normal and pathological ears. Each ratio of the number of basal and apical ganglion cells in groups 2, 3, 4, and 5 to the mean number in group 1 (basal and apical survival ratios) was at least approximately 40%. There was no statistical difference between these two ratios in groups 2, 3, 4, and 5. The mean ($\pm$ SD) total number of ganglion cells in ears with documented profound deafness was $15,417 \pm 5,944$, which is approximately 40% of those present in normal ears. Our results suggest that normally, cochlear neurons are completely present at birth and minimally regress during the first decade of life. In addition, although intergroup differences among various pathological groups were present, the majority of pathological ears had more than 10,000 spiral ganglion cells present. Cochlear implantation has gradually been recognized as an effective and reliable tool for rehabilitation of children who have profound deafness, even congenitally or prelingually deafened children. On the basis of the results obtained in this study, we discuss the implications for cochlear implantation in children.

KEY WORDS — child, cochlear implantation, spiral ganglion cell, temporal bone histopathology.

INTRODUCTION

Cochlear implantation has gradually been recognized as an effective and reliable tool for rehabilitation of children who have profound deafness. Congenitally or prelingually deafened children, as well as those deafened postlingually, demonstrate remarkable speech perception performance following cochlear implantation.¹⁻⁴ Several investigators propose that early implantation in congenitally deafened children provides better language acquisition results.^{1,2,4} The dramatic results achieved in children younger than 2 years of age have been the impetus for lowering the implantation criteria to 1 year of age.⁵ Although numerous variables influencing the results of speech recognition after implantation are not well understood, the degree of survival of spiral ganglion cells in cochlea is considered to be of primary importance.⁶

There have been several quantitative studies of the human spiral ganglion cells in adults with normal and pathological ears, including those with profound deaf-

ness.⁷⁻¹⁴ Although some of those studies include a few cases of children under 10 years of age,^{7,9-11} to our knowledge, there is no systematic survey to determine whether spiral ganglion cell populations differ among children with different pathological conditions or whether the postnatal change of ganglion cell populations is present during childhood. The purpose of this study was to quantitatively analyze spiral ganglion cell populations in ears of children with different pathological conditions and to compare these features with data from age-matched control ears.

MATERIALS AND METHODS

This study is based upon analysis of a series of histologic sections of 63 human temporal bone specimens from 43 individuals, ranging in age at the time of death from 4 days to 9 years. We divided these specimens into 5 diagnostic groups: group 1 (control group), temporal bones obtained from individuals without any known congenital anomaly or clinical history or pathological evidence of ear disease; group 2, temporal bones from individuals with con-

From the Elizabeth McCullough Knowles Otopathology Laboratory, Division of Otopathology (Miura, Sando, Orita), and the Division of Otolaryngology (Hirsch), Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. Supported by Research Grant R01DC00123-24 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

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TABLE 1. DIAGNOSIS, NUMBER, AND AGE OF FIVE GROUPS

Group	Diagnosis	No. of Individuals	Mean Age at Time of Death (mo)	No. of Temporal Bones
1	Control	13	40.2 ± 37.2	13
2	Congenital infectious disease	7 (3)	12.9 ± 21.5 (28.3 ± 27.5)	13 (5)
3	Chromosomal aberration	7	6.2 ± 13.2	11
4	Hereditary or genetic anomaly	12 (5)	33.5 ± 29.9 (26.6 ± 16.4)	21 (9)
5	Perinatal or postnatal asphyxia	4 (3)	13.9 ± 9.7 (18.3 ± 4.9)	5 (4)
Total		43 (11)	25.9 ± 30.2 (24.8 ± 16.8)	63 (18)

Data in parentheses apply to individuals with documented profound deafness.

genital infectious diseases, including congenital rubella and cytomegalovirus infection; group 3, temporal bones from individuals with chromosomal aberrations, including trisomies 13, 18, and 21; group 4, temporal bones from individuals with multiple craniofacial anomalies with hereditary or genetic causes, including Noonan's syndrome, osteopetrosis, Waardenburg's syndrome, Pelizaeus-Merzbacher disease, Alagille syndrome, Apert's syndrome, Beckwith-Wiedemann syndrome, and DiGeorge syndrome; and group 5, temporal bones from individuals with perinatal or postnatal asphyxia requiring long-term oxygen therapy. The number of individuals, the number of temporal bones, and the mean age at the time of death in each group are summarized in Table 1. Eighteen of the 63 ears had documented profound deafness confirmed by auditory brain stem response testing and/or behavioral audiometry. They belonged to groups 2, 4, and 5. The number of patients, the number of temporal bones, and the mean age at the time of death are also presented in Table 1. The results obtained from the 18 ears with documented profound deafness are displayed separately from the data from all specimens. With regard to the hearing status of the remaining ears without documented profound deafness, we can show that 1 ear in group 1 had normal hearing as confirmed by a pure tone audiogram. However, the other ears had no documented hearing status.

The specimens were fixed in 10% formalin, decalcified in 5% trichloroacetic acid, dehydrated in graded concentrations of ethanol, and embedded in celloidin. The celloidin blocks were sectioned horizontally at 20 μ m. Every 10th section was stained with hematoxylin and eosin and prepared for light microscopic

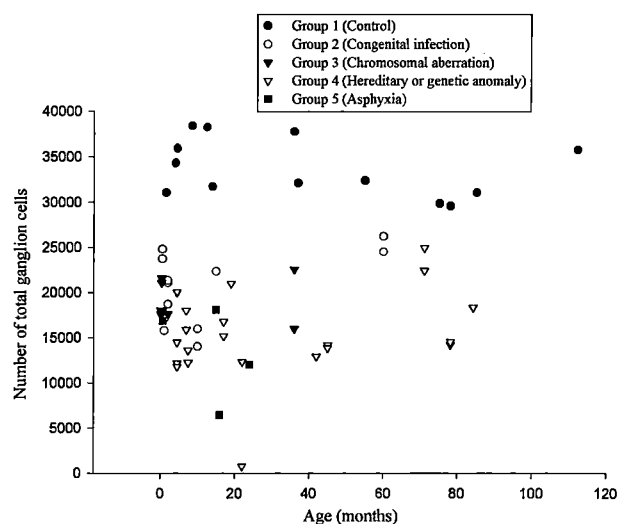


Fig 1. Relationship between age (months) and total spiral ganglion cell numbers in 5 diagnostic groups. Note that some plotted data points with very close values in groups 2 through 5 almost overlap.

study. The population of spiral ganglion cells was estimated by the method described by Otte et al⁷ and Nadol et al.⁸ According to this method, the spiral ganglion cells were divided into 4 segments by a vertical midmodiolar line. For a 32-mm-long cochlea, these segments provide innervation for the organ of Corti approximately as follows: segment 1, 0 to 6 mm; segment 2, 6 to 15 mm; segment 3, 15 to 22 mm; and segment 4, 22 to 32 mm.⁷ The ganglion cells with visible nucleoli in each segment were counted with the aid of an ocular grid. The counts were multiplied by 10 to account for unmounted sections, and also by a factor of 0.9 to account for doubly counted spiral ganglion cells.¹⁵ The ganglion cell populations were determined for each of the 4 defined segments. The total number of ganglion cells was summed for all 4 segments of the cochlea. In our study, the basal half of the ganglion cells represents segments 1 and 2, and the apical half of the ganglion cells represents segments 3 and 4.

RESULTS

Figure 1 shows the total number of ganglion cells in each ear for all 5 diagnostic groups as a function of age. Linear regression analysis indicated that the total number of ganglion cells did not vary significantly with age in each of the 5 groups (group 1, $r^2 = 0.128$, $F_{1,11} = 1.62$, $p = .229$; group 2, $r^2 = 0.243$, $F_{1,11} = 3.52$, $p = .087$; group 3, $r^2 = 0.011$, $F_{1,9} = 0.96$, $p = .764$; group 4, $r^2 = 0.091$, $F_{1,19} = 1.91$, $p = .183$; and group 5, $r^2 = 0.146$, $F_{1,3} = 0.514$, $p = .525$). These findings suggest that the total number of ganglion cells did not change as a function of age during the first 10 years in either normal or variously pathological ears.

TABLE 2. NUMBER OF TOTAL AND SEGMENTAL SPIRAL GANGLION CELLS AMONG FIVE GROUPS

Group	No. of Specimens	Segment 1	Segment 2	Segment 3	Segment 4	Total
All specimens						
1	13	4,382 ± 997	13,127 ± 1,367	7,714 ± 1,232	8,476 ± 1,327	33,702 ± 3,190
2	13	2,136 ± 1,055	8,077 ± 1,782	4,803 ± 1,240	5,507 ± 1,392	20,523 ± 3,892
3	11	2,203 ± 831	8,116 ± 1,248	4,716 ± 703	3,845 ± 1,139	18,880 ± 2,088
4	21	1,630 ± 803	6,270 ± 1,998	3,643 ± 1,519	3,674 ± 1,486	15,218 ± 4,926
5	5	1,370 ± 1,025	5,933 ± 2,621	3,271 ± 1,643	3,728 ± 1,184	14,301 ± 5,073
Total	63	2,382 ± 1,388	8,353 ± 3,115	4,880 ± 2,001	5,077 ± 2,296	20,693 ± 8,038
One specimen from each individual						
1	13	4,382 ± 997	13,127 ± 1,367	7,714 ± 1,232	8,476 ± 1,327	33,702 ± 3,190
2	7	2,195 ± 1,018	8,266 ± 1,788	4,881 ± 1,287	5,304 ± 1,549	20,645 ± 3,496
3	7	2,136 ± 764	8,267 ± 1,254	4,792 ± 775	3,725 ± 1,151	18,919 ± 2,040
4	12	1,623 ± 817	6,569 ± 1,567	3,616 ± 973	3,654 ± 1,224	15,462 ± 3,538
5	4	1,258 ± 1,147	5,659 ± 2,942	2,846 ± 1,549	3,580 ± 1,312	13,343 ± 5,309
Total	43	2,560 ± 1,508	9,020 ± 3,268	5,180 ± 2,102	5,385 ± 2,477	22,186 ± 8,610
Specimens with documented profound deafness						
2	5	2,678 ± 1,275	8,861 ± 2,260	4,658 ± 1,671	4,415 ± 1,243	20,614 ± 5,351
4	9	1,509 ± 768	5,485 ± 1,936	3,244 ± 1,233	3,072 ± 1,638	13,310 ± 5,051
5	4	1,544 ± 1,095	5,477 ± 2,788	2,880 ± 1,607	3,762 ± 1,364	13,662 ± 5,620
Total	18	1,843 ± 1,076	6,421 ± 2,600	3,556 ± 1,531	3,599 ± 1,518	15,417 ± 5,944

Data are mean ± SD.

The means and standard deviations of the total and 4 segmental spiral ganglion cell populations among the 5 diagnostic groups (for all specimens) are presented in Table 2. Analysis of variance demonstrated that the total number of ganglion cells significantly differed among the 5 groups ($F_{4,58} = 47.58$, $p < .0001$). Post hoc testing (Bonferroni) indicated that the total number of ganglion cells was significantly larger in group 1 than in each of groups 2, 3, 4, and 5 ($p < .01$), and that the number was significantly larger in group 2 than in each of groups 4 and 5 ($p < .01$ and $p < .05$, respectively; Fig 2). It is important to note that the single ear with DiGeorge syndrome (within group 4) exhibited an extremely small number of total ganglion cells (747 counts; Figs 1 and 3).

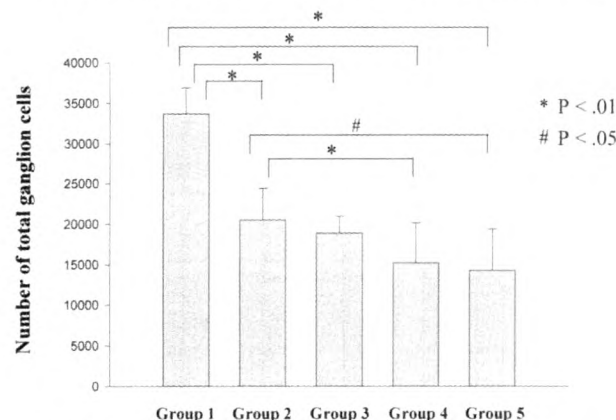


Fig 2. Means and standard deviations of total spiral ganglion cell numbers in 5 diagnostic groups. Mean number in group 1 (control ears) was 33,702. Symbols * and # designate groups with significantly different mean values.

Analysis of each of the 4 segmental ganglion cell populations led to the same conclusions, except that post hoc testing (Bonferroni) showed the ganglion cell population in each of segments 1, 2, and 3 to be significantly larger in group 1 than in each of groups 2, 3, 4, and 5 ($p < .01$), but analysis of comparable segments among groups 2 through 5 gave results that did not differ significantly. Meanwhile, in the present study, in 20 cases, the same individual contributed both ears to the sample population to increase the number of each diagnostic group. However, there is a possibility that this sampling method may bias the statistical results regarding the intergroup differences.

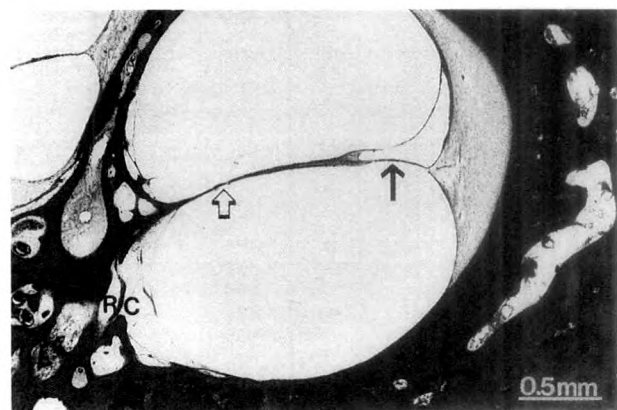


Fig 3. Photomicrograph of right temporal bone, obtained from 22-month-old boy with DiGeorge syndrome (within group 4). Basal turn of cochlea is shown. Spiral ganglion cells are almost absent in poorly developed Rosenthal's canal (RC). Note that organ of Corti is severely collapsed (black arrow), and that dendritic nerve fibers are also absent in thin osseous spiral lamina (white arrow).

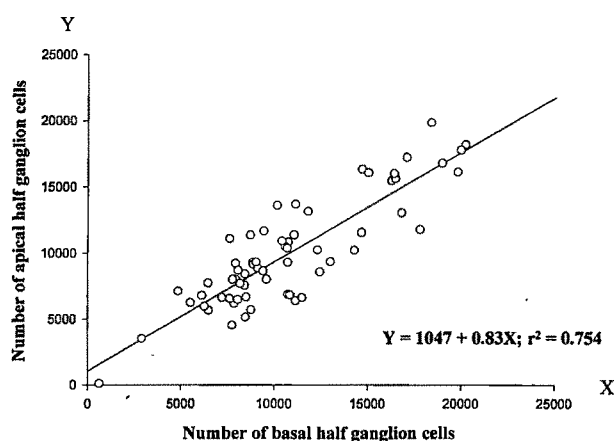


Fig 4. Relationship between basal half and apical half of ganglion cell numbers for all data.

Therefore, the analysis was repeated by choosing 1 temporal bone at random from each individual with the use of a table of random digits. The means and standard deviations of the total and 4 segmental spiral ganglion cell populations among the 5 diagnostic groups (1 temporal bone from each individual; 43 ears) are also presented in Table 2. These values in each diagnostic group were very similar to the data from all the specimens (63 ears). Analysis of variance demonstrated that the total number of ganglion cells significantly differed among the 5 groups ($F_{4,38} = 18.05$, $p < .0001$). Post hoc testing (Bonferroni) indicated that the total number of ganglion cells was significantly larger in group 1 than in each of groups 2, 3, 4, and 5 ($p < .01$), and that the number was significantly larger in group 2 than in each of groups 4 and 5 ($p < .05$). Hence, using more than 1 temporal bone from each individual to maximize the available number of specimens was estimated to minimally affect the statistical analysis regarding the intergroup differences.

In addition, regression analysis revealed that the number of the apical half of the ganglion cells linearly increased as a function of the number of the basal half of the ganglion cells ($r^2 = 0.754$, $F_{1,61} = 187.34$, $p < .001$, for all specimens; Fig 4). This finding suggests a strong correlation between the apical and basal ganglion cell populations in both normal and pathological ears. Then, since regression analysis demonstrated that the number of either the basal or apical half of the ganglion cells in control ears (group 1) did not change significantly with age ($r^2 = 0.009$, $F_{1,11} = 0.098$, $p = .760$ and $r^2 = 0.219$, $F_{1,11} = 3.08$, $p = .107$), each mean number in group 1 provides a standard population during childhood (from birth to 9 years of age). We then analyzed the ratio of the number of the basal ganglion cells in each ear from groups 2, 3, 4, and 5 to the mean number in group 1 and derived a basal survival ratio. Similarly,

TABLE 3. BASAL AND APICAL SURVIVAL RATIOS OF SPIRAL GANGLION CELLS AMONG FOUR PATHOLOGICAL GROUPS

Group	No. of Specimens	Basal Survival Ratio (%)	Apical Survival Ratio (%)	Difference Between Basal and Apical Survival Ratios
All specimens within four pathological groups				
2	13	58.3 ± 14.2	63.7 ± 13.4	NS
3	11	58.9 ± 6.2	52.9 ± 9.7	NS
4	21	45.1 ± 13.9	45.2 ± 17.1	NS
5	5	41.7 ± 19.5	43.2 ± 13.6	NS
Total	50	51.3 ± 14.8	51.5 ± 16.2	NS
Specimens with documented profound deafness				
2	5	65.9 ± 19.3	56.0 ± 12.8	NS
4	9	39.9 ± 14.3	39.0 ± 16.6	NS
5	4	40.1 ± 22.1	41.0 ± 14.7	NS
Total	18	47.2 ± 20.3	44.2 ± 16.2	NS

Basal and apical survival ratios were defined as ratios of number of basal- and apical-half ganglion cells to each of mean numbers in group 1 (control specimens), respectively. Data are mean ± SD.
NS — not significant.

the ratio of the number of the apical ganglion cells in each ear of groups 2 through 5 to the mean number in group 1 (apical survival ratio) was evaluated. The means and standard deviations of these 2 ratios in groups 2 through 5 are presented in Table 3. Paired *t*-tests indicated that there was no statistical difference between the 2 ratios in groups 2, 3, 4, and 5.

In addition, we separately analyzed the ganglion cell populations in ears with documented profound deafness. The means and standard deviations of the total and 4 segmental spiral ganglion cell populations in ears with profound deafness are also presented in Table 2. Unpaired *t*-tests revealed that the total number of ganglion cells in ears with documented profound deafness ($15,417 \pm 5,944$) was significantly smaller than the ganglion cell population in ears without documented deafness ($18,376 \pm 3,639$) within groups 2 through 5 (ie, pathological groups; $t = 2.19$, $p < .05$). Figure 5 shows the total number of ganglion cells in each ear with documented profound deafness as a function of age. There was a significant intergroup difference in the total number of ganglion cells between groups 2 and 4 (unpaired *t*-test, $t = 2.54$; $p < .05$). In addition, regression analysis revealed that the number of the apical half of the ganglion cells linearly increased as a function of the number of the basal half of the ganglion cells ($r^2 = 0.707$, $F_{1,16} = 38.538$, $p < .001$, for 18 specimens with documented profound deafness; Fig 6). The means and standard deviations of the basal and apical ratios in ears with documented profound deafness are also pre-

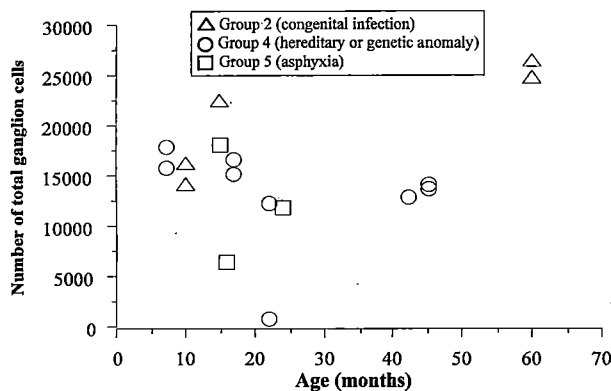


Fig 5. Relationship between age (months) and total spiral ganglion cell numbers in ears with documented profound deafness in different groups (groups 2, 4, and 5). Note that 2 data points with very close values in group 5 (18,099 and 18,135) almost overlap.

sented in Table 3. Paired *t*-tests indicated that there was no statistical difference between the 2 ratios.

DISCUSSION

The spiral ganglion cell population is considered to decrease with age. Furthermore, the loss is reported to begin at an early age and continue throughout life.¹⁶ Otte et al⁷ analyzed 100 "hearing ears" from subjects without known disease directly affecting the spiral ganglion cells. They reported that the total ganglion cell population ranged from 36,913 for the first decade to 18,626 for the ninth decade, with a uniform progressive loss of approximately 2,000 ganglion cells per decade. Their result for the first decade, however, was based upon analysis from 2 subjects between 9 and 10 years of age. Hinojosa et al¹⁰ also reported that the spiral ganglion cell population from a 9-year-old boy with normal hearing was 38,352 cells. However, to our knowledge, there is no systematic survey to describe the postnatal change of ganglion cells during childhood. Our present study demonstrated that the total spiral ganglion cell population in 13 normal ears (from 13 individuals) did not change significantly during the period from birth to 9 years of age, and that the mean population was 33,702 cells. These results suggest that normally, cochlear neurons are completely present at birth and regress minimally during the first decade of life.

Our results showed that the spiral ganglion cell population was significantly larger in the control group than in each of the 4 pathological groups, and that there were significant intergroup differences between group 2 (congenital infectious disease) and each of groups 4 (hereditary or genetic anomaly) and 5 (prenatal and postnatal asphyxia). A similar intergroup difference was present among ears with documented profound deafness. Several investigators reported that spiral ganglion cell populations varied

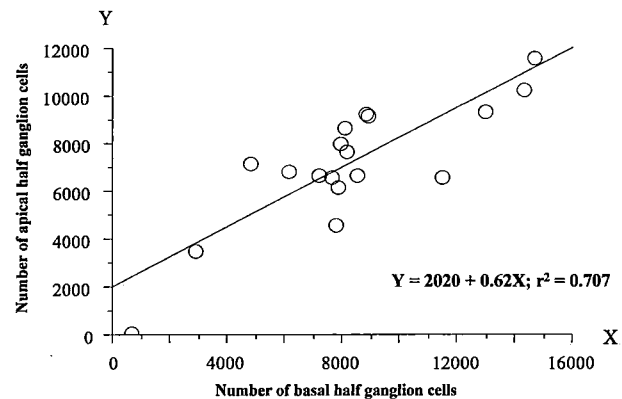


Fig 6. Relationship between basal half and apical half of ganglion cell numbers for 18 specimens with documented profound deafness.

among adults with different otopathological conditions.^{7,8,11,13,14} Since their studies were based on analysis of subjects whose ages varied widely, the decrease of ganglion cells caused by aging influenced their results. In contrast, the present study revealed that spiral ganglion cell populations did not change significantly with age during childhood in either normal or pathological ears.

Zimmermann et al,¹⁷ analyzing 12 pathological specimens from 10 individuals over 50 years of age, reported that both primary degeneration and secondary (or retrograde) degeneration of spiral ganglion cells were more severe in the basal half than in the apical half of the cochlea. Other investigators have reported a similar predilection for degeneration of spiral ganglion cells to occur in the basal turn of the cochlea in adult cases.^{7-9,16,18} This predilection may partly be explained by the fact that the loss of ganglion cells with advancing age is predominant in the basal part of the cochlea.¹⁶ On the other hand, Vasama and Linthicum¹⁹ reported that apical ganglion cells were significantly more affected than basal ganglion cells in adults with idiopathic sudden sensorineural hearing loss. They believed that apical ganglion cells in humans may be less resistant to neurotrophic viruses that attack the inner ear, including herpes, mumps, and measles, than basal ganglion cells. In contrast, the results from our analysis of pediatric cases did not identify such a predominantly greater loss of ganglion cells to occur in either the basal or apical half of the cochlea in patients with diverse pathological conditions. In addition, there was a strong correlation between ganglion cell populations in these 2 cochlear segments, regardless of the presence or absence of pathological conditions. In ear disorders with congenital or perinatal causes, spiral ganglion cells were equally affected within the cochlea.

There has not been conclusive evidence proving that the spiral ganglion cell population surviving in

the cochlea has a direct effect on speech perception in patients with cochlear implants. Fayad et al,²⁰ analyzing 16 temporal bones with single-channel cochlear implants from 13 individuals, reported that there was no association between the number of spiral ganglion cells and clinical performance. They believed that the response to stimulation might occur with as little as 10% of the normal population of spiral ganglion cells. On the basis of previously published articles, Blamey²¹ also inferred that the minimum number of ganglion cells required for satisfactory speech perception in cochlear implant recipients was approximately 5,000. In each of the pathological groups in our study, the mean number of total ganglion cells was at least 40% of the control mean, as was the mean number in ears with documented profound deafness. It can be seen that the majority of our cases would exceed the minimum requirement of ganglion cells for cochlear implantation. It is also important to note that the spiral ganglion cells in the basal half, which seems to be more crucial to cochlear implantation, were well preserved. These results provide objective support for the success of cochlear implantation for most pediatric candidates regardless of the cause of hearing loss. However, we should be

aware that the presence of spiral ganglion cells observed by a light microscope does not guarantee normal physiological function during life.^{8,13} In addition, we should also take into consideration that spiral ganglion cells might be almost absent from the inner ears of a small minority of pathological cases.

Success in cochlear implantation in congenitally or prelingually deafened children may depend on many factors, including the integrity of the auditory nerve and central auditory pathway, residual hearing, age at implantation, age at onset of deafness, duration of deafness, length of implant use, and preoperative level of oral language.^{1-5,22} Many children in the pathological groups categorized in this study have secondary or multiple handicaps besides hearing loss, including cognitive disabilities and behavior disorders. If they become candidates for cochlear implantation, the outcome is likely poorer than it would be in otherwise healthy deafened children. A recent study, however, indicated that even children with multiple handicaps obtained substantial benefit from cochlear implantation.²³ Our results in this study provide theoretical and laboratory support for cochlear implantation in these deafened children.

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The Department of Otolaryngology–Head and Neck Surgery at the Hospital Europeen Georges Pompidou, University of Paris V, France, together with the association Progres 2000, is sponsoring a traveling fellowship starting May 1, 2003. A 2-month scholarship will provide extensive training in laryngology–head and neck surgery and oncology every year during the months of May and June. The successful candidate will be an active member in the department and participate in research. For further information, contact ollivier.laccourreye@hop.egp.ap-hop-paris.fr.

NEW TREATMENT OPTIONS FOR LYMPHANGIOMA IN INFANTS AND CHILDREN

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Lymphangiomas are congenital malformations of the lymphatic system. These lesions occur most often in the head and neck area, and their treatment continues to be a challenge. Fortunately, a number of advances have occurred in the diagnosis and management of lymphatic malformations in the past decade. The purpose of this article is to clarify the embryology, pathogenesis, histopathology, and classification of these lesions, as well as to describe their various forms of clinical presentation. We provide a complete review of the diagnostic measures available and thoroughly discuss new therapeutic interventions proposed to treat lymphangiomas.

KEY WORDS — cervicofacial malformation, congenital malformation, cystic hygroma, head and neck tumor, lymphangioma, lymphovenous malformation, OK-432.

EMBRYOLOGY AND PATHOGENESIS

Lymphangiomas are localized malformations in the development of the lymphatic system that most commonly occur in the head and neck. They were first described in 1828 by Redenbacker,¹ who coined the term *ranula congenita*. To understand their pathogenesis, one must be familiar with the embryology of the lymphatic system.

Early in the 20th century, Sabin² discovered that lymphatic structures arise from 5 primitive buds that sprout from the venous system. By the seventh embryonic week, paired jugular sacs, paired posterior sacs, and a single retroperitoneal sac form. The jugular sacs, located next to the jugular veins, develop first. Sabin proposed that endothelial sproutings from these sacs extend centrifugally into the embryo to form the peripheral lymphatic system. McClure and Huntington, in contrast, advocated centripetal development of the lymphatic system from the confluence of peripheral mesenchymal spaces that anastomose with the venous system centrally.^{3,4} A combined venous-mesenchymal theory was proposed by van der Gagt and Kutsuna.⁴ Today, the most accepted theory is that of Sabin, which also is supported by the work of van der Putte.⁵

Although the precise cause of congenital lymphangiomas remains unknown, it probably reflects a combination of failure of lymphatics to connect with the venous system, abnormal budding of lymphatic structures, and abnormal sequestration of lymphatic tissue early in embryogenesis.^{4,6-8} Lymphangiomas also

may be acquired after surgical procedures, minor trauma, infection, chronic inflammation, or obstruction of lymphatic drainage.

HISTOPATHOLOGY AND CLASSIFICATIONS

Lymphangiomas are classified traditionally into 3 categories based on histologic appearance: 1) capillary lymphangiomas, containing a network of capillary-sized lymphatic channels that tend to involve the epidermis; 2) cavernous lymphangiomas, composed of dilated lymphatics that infiltrate surrounding tissue; and 3) cystic hygromas, macroscopic unilocular or multilocular cystic masses lined by a single layer of endothelium supported by a connective tissue stroma and containing straw-colored, proteinaceous fluid.^{4,8-11} A fourth category, hemangiolymphangioma, was proposed in 1975 and refers to lymphangiomas that also have a vascular component.¹² All types of lymphangiomas are manifestations of the same pathological process and often coexist within a single lesion.⁴

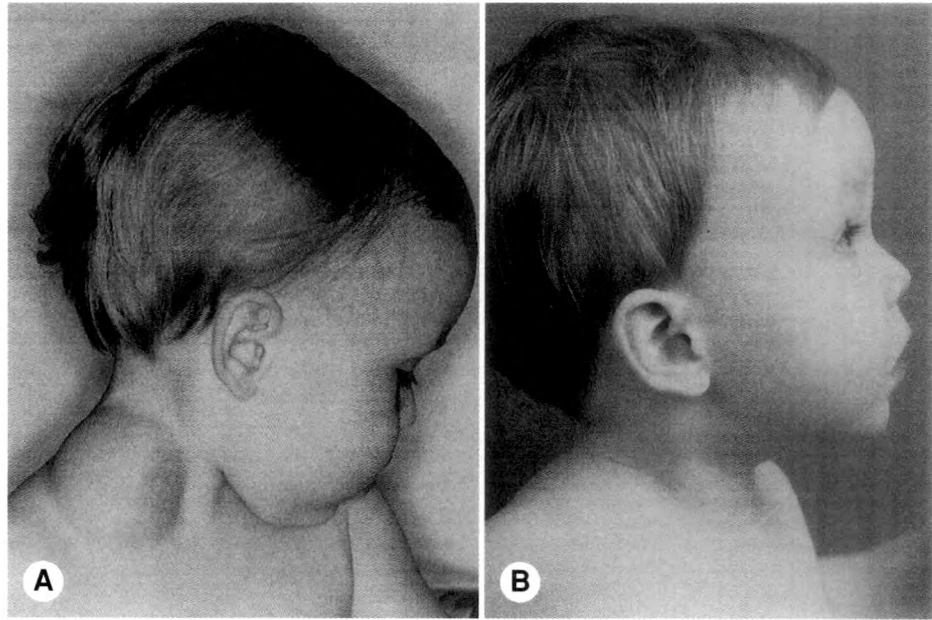
An alternative classification that is not histologically based but is, rather, therapeutically based was proposed by Smith et al to reflect responses seen to sclerotherapy.^{13,14} Lymphangiomas are classified as macrocystic, microcystic, or mixed. Macrocystic lesions are cystic spaces of at least 2 cm³, microcystic lesions are cystic spaces of less than 2 cm³, and mixed lesions contain both macrocystic and microcystic components.¹³⁻¹⁵

A third classification was proposed by de Serres

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Fig 1. Patient with stage I lymphangioma of right side of his neck. Photographs taken A) before and B) after 1 injection of OK-432 demonstrate complete response to this treatment.



et al,¹⁶ together with a staging system based on disease extent and location. The following stages are recognized: stage I, unilateral infrahyoid disease; stage II, unilateral suprahyoid disease; stage III, unilateral suprahyoid and infrahyoid disease; stage IV, bilateral suprahyoid disease; and stage V, bilateral suprahyoid and infrahyoid disease¹⁶ (Fig 1). In clinical practice, using a combined Smith-de Serres classification is most useful.

CLINICAL PRESENTATION

Most lymphangiomas are diagnosed in infancy. More than 50% are recognized at birth, and 90% are detected before the age of 2 years.^{17,18} The majority present in the head and neck area, and they affect both sexes equally.^{7,19} Usually, the mass is asymptomatic and involves the anterior or posterior cervical triangle.¹⁹ Lymphangiomas of the anterior triangle may extend into the submandibular and submental spaces and infiltrate the floor of the mouth.^{20,21}

Lymphangiomas are soft and "doughy" on palpation, varying in size from a few millimeters to more than 30 cm. The overlying skin is usually unremarkable; however, it can be thin, atrophic, or bluish.²¹ When the tongue is affected, its mucosal surface may be covered with small, whitish vesicles. Although lymphangiomas are inherently slow-growing, sudden enlargement may result from spontaneous or traumatic hemorrhage into cystic spaces or after an upper respiratory tract infection.²²

The symptoms are related to the size, location, and extent of the lymphangioma.⁷ Respiratory compromise is the most significant complication of head and neck lesions and is commonly seen in patients with

large masses of the neck or mediastinum. Infiltration of the floor of the mouth, base of the tongue, pharynx, or laryngeal structures also may result in dysphonia, stridor, apnea, cyanosis, or life-threatening dyspnea. Many patients present with feeding difficulties and subsequent failure to thrive.²³ Suprahyoid disease tends to cause more breathing difficulties and dysphagia than does infrahyoid disease.²⁴ Pain is not a common manifestation unless infection is present, but disfigurement can be significant.

When lymphatic malformations extend into the mediastinum, the presenting signs and symptoms may include pleural and/or pericardial effusions with chronic pain or pathological fractures resulting from involvement of rib or thoracic vertebral bone.²⁵ Severe osteolysis, an extremely rare but life-threatening complication, may occur when lymphangiomas infiltrate and cause subsequent resorption of adjacent bone. Dissolution of bone caused by either lymphangiomas or hemangiomas is termed Gorham-Stout syndrome.²⁶

Lymphangiomas are commonly associated with Turner syndrome and in these patients are frequently diagnosed before birth by ultrasonography (US). Other syndromic associations include Klinefelter, Noonan, Fryns, and several of the trisomies.^{6,9,21} Lymphangiomas also have been related to exposure to teratogens such as alcohol.²⁷

DIAGNOSIS

A detailed history and physical examination are essential. These should be complemented by pharyngolaryngoscopy in all patients in whom there is a suspicion of pharyngeal or laryngeal involvement. An imaging study such as US, computed tomogra-

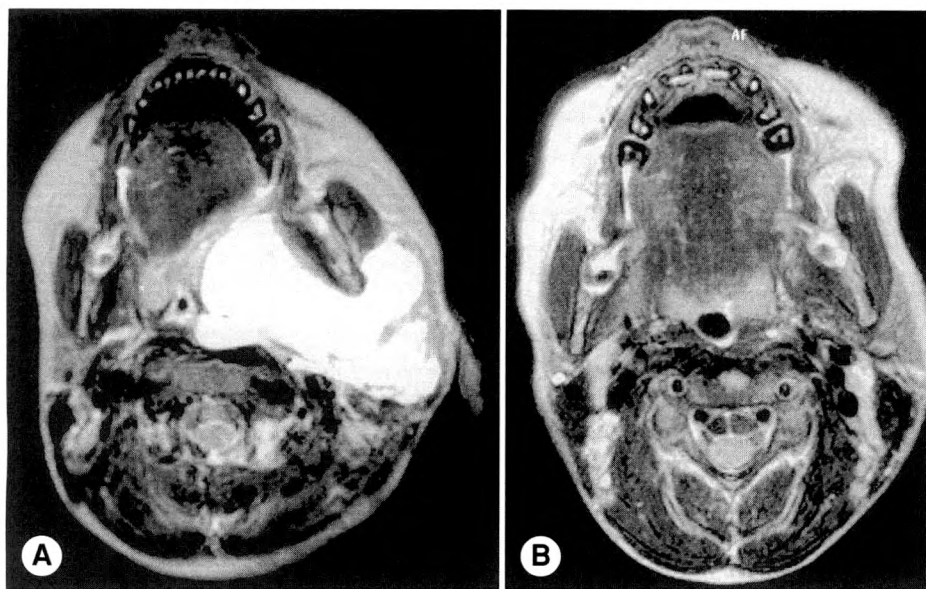


Fig 2. Magnetic resonance imaging scans. **A)** Extensive parapharyngeal lymphangioma. Lesion borders are well defined. **B)** Complete resolution of lesion following 2 injection treatments of OK-432.

phy (CT), or magnetic resonance imaging (MRI) is also essential.

The US pattern of lymphangiomas is well described.⁹ Typically, lesions appear as hypoechoic, multilocular cystic masses with septa of variable thickness. Solid echogenic components correspond to collections of small abnormal lymphatic channels. Ultrasonography is particularly useful in demonstrating the presence of lymphangiomas and determining relationships to major adjacent structures; however, it is limited in its ability to define mediastinal and retropharyngeal extension.^{9,28}

By CT, lymphangiomas appear as thin-walled, nonenhancing, homogeneous cystic masses that have the same density as water. Use of intravenous contrast medium may result in enhancement of the cyst wall and better definition of the anatomic location of the malformations with respect to blood vessels. Although CT offers excellent visualization of lesions extending into the mediastinum, deep neck structures, and retropharynx, it is often associated with bony artifact from the skull base and vertebrae. Furthermore, it may be difficult to distinguish a mass from surrounding soft tissue of similar attenuation.²⁸

Magnetic resonance imaging is the imaging method of choice for lymphangiomas. Its utility was first reported by Siegel et al²⁹ in 1989. Lesions are typically hypointense on T1-weighted images and hyperintense on T2-weighted images.³⁰ The use of contrast medium (gadolinium tetra-azacyclododecane tetra-acetic acid solution) helps to differentiate between deep hemangiomas and lymphangiomas.³¹ When spontaneous or traumatic hemorrhage has occurred in cystic cavities, fluid-fluid levels can be readily appreciated. The advantages of MRI over US and

CT include superior multiplanar capabilities and lack of bony artifact. It is also superior to US and CT in accurately defining lesion borders and in evaluating the extent of disease³² (Fig 2).

Chest radiographs remain very useful in ruling out mediastinal extension or pleural effusions.^{23,25} Similarly, barium swallow studies remain valuable in assessing esophageal compression. Although lymphangiomas can be transilluminated, this technique is not reliable, nor does it help determine the extent of disease.²³ Masses that are infected and those containing a large vascular component or multiple septations do not transilluminate.

Fetal cervical cystic hygromas can be visualized by abdominal US as early as 10 weeks' gestational age.³³ Transvaginal US is superior to traditional abdominal US, because it shows more detail and allows earlier detection of fetal cystic hygromas. Elevated levels of alpha-fetoprotein in maternal serum and amniotic fluid have been found in pregnancies associated with lymphangiomas.²⁷ When lymphatic malformations are detected on prenatal evaluation, a detailed US review of fetal anatomy must be performed to rule out structural malformations. Some authors also recommend a fetal karyotype analysis in the presence of a cystic hygroma.^{6,8}

TREATMENT OPTIONS

A tracheotomy should be performed, regardless of the treatment method, when a patient's airway is compromised by a large lesion that extends into the tongue, floor of the mouth, epiglottis, or glottic area.

Observation. The incidence of spontaneous regression of cystic hygromas is reported to be as high as 15%.^{7,34} On this basis, many investigators recom-

ment withholding treatment for a period of time.^{34,35} This conservative option should only be considered in the absence of symptoms. One also must keep in mind the potential risk of acute enlargement of the mass following infection or hemorrhage. A period of observation may be warranted for infiltrating, microcystic lesions involving the parotid gland to allow for growth prior to therapeutic intervention in order to minimize the risk of injury to the facial nerve. Some authors who are skeptical of the possibility of spontaneous involution recommend immediate treatment.⁸

Aspiration. Previously regarded as a rapid, safe, and minimally invasive treatment for lymphangiomas, percutaneous aspiration is now rarely indicated. This procedure is usually followed by recurrence, hemorrhage, or infection.^{7,23} However, if there is acute enlargement of a lesion with airway compromise, aspiration for emergency decompression may be an effective temporizing measure.^{10,36}

To prevent the development of polyhydramnios, permanent facial deformity, and hydrops fetalis, Chen et al³⁷ advocate serial intrauterine aspirations of large, prenatally diagnosed cystic hygromas. Intrauterine cyst decompression by this technique has been reported to facilitate vaginal delivery of affected infants who would have otherwise required cesarean section deliveries.

Surgery. Many authors agree that complete surgical excision is the treatment of choice for lymphangiomas.^{7,16,17,38} However, the infiltrating nature of these lesions and the involvement of vital structures in the head and neck area make total extirpation nearly impossible in most cases. Because these lesions are benign, major vessels and significant nerves should not be sacrificed. This limitation makes the dissection tedious and difficult. Often, staged excision is done for extensive disease; however, removal of a portion of the disease burden may lead to the enlargement of cysts in neighboring unresected regions.^{7,16} Total removal of the tumor is usually successful with small lesions that do not involve the oral cavity. With invasive tumors larger than 5 cm or involving the upper aerodigestive tract, complete removal is unlikely to be possible without significant functional impairment.^{8,38} Recurrence rates following "complete" excision range from 0% to 27%; after partial excision, the recurrence rate is 50% to 100%.^{7,10,19,38,39} Most recurrences occur within the first year of resection, but delayed recurrences as long as 10 years after the initial operation have been reported.³⁹

Timing of the initial surgery continues to be debated. If observation is first chosen as a feasible and

safe option, the question then arises as to when to perform surgery. According to some authors, excision of cervical lymphangiomas should be recommended only if there is no regression by the age of 5 years.³⁵ Others recommend earlier operative removal to avoid the complications of potential infection, which may render surgery considerably more difficult. Excision should be delayed at least 3 months after an acute infection.²⁰

Complications from surgical excision are well documented, and their rate varies from 10% to 33%.³⁹ Intraoperative blood loss may be significant, especially in young children.⁴⁰ Muscle weakness, Horner's syndrome, Frey's syndrome, recurrent airway obstruction, life-threatening infections such as mediastinitis and sepsis, chylothorax, and death are some of the more serious postoperative complications. Nerve paralysis occurs in as many as one third of the cervicofacial lymphangioma cases treated surgically.⁷ The most commonly injured nerve is the marginal mandibular branch of the facial nerve — a complication usually seen in patients with parotid, buccal, or submandibular gland involvement.¹⁶ Injury to the facial nerve may leave the patient with serious functional, aesthetic, and psychological sequelae.

Cranial nerves IX through XII, the cervical sympathetic chain, and the brachial plexus also are vulnerable structures and may be damaged during surgical removal of cervical lymphangiomas.¹⁰ The risk of intraoperative nerve injury is related to the size and extent of the lesion, rather than to the child's age at the time of surgery.⁷ Scarring and large tissue defects are some of the possible cosmetic consequences related to surgical excision of lymphangiomas.

Several authors have reported an increased incidence of surgery-related complications in patients with suprahyoid as compared to infrahyoid disease.^{7,16} Laterality also appears to play a role in the surgical complication rate; patients with bilateral lesions experience more postoperative problems than do those with unilateral lesions.¹⁶

Laser Treatment. The use of laser technology, both argon beam and CO₂, has been reported to be effective in the treatment of lymphangiomas for debulking or complete excision.^{34,41} Lasers are most useful in ablating or excising lymphangiomas of the larynx and tongue base (Fig 3). The advantages over traditional surgery include less postoperative edema, less tissue trauma, and less blood loss. However, the rate of recurrence is high, since it is nearly impossible to completely eradicate the disease burden, and multiple treatments are often necessary.

Magnetic resonance-controlled laser-induced interstitial thermotherapy (LITT) has been proposed

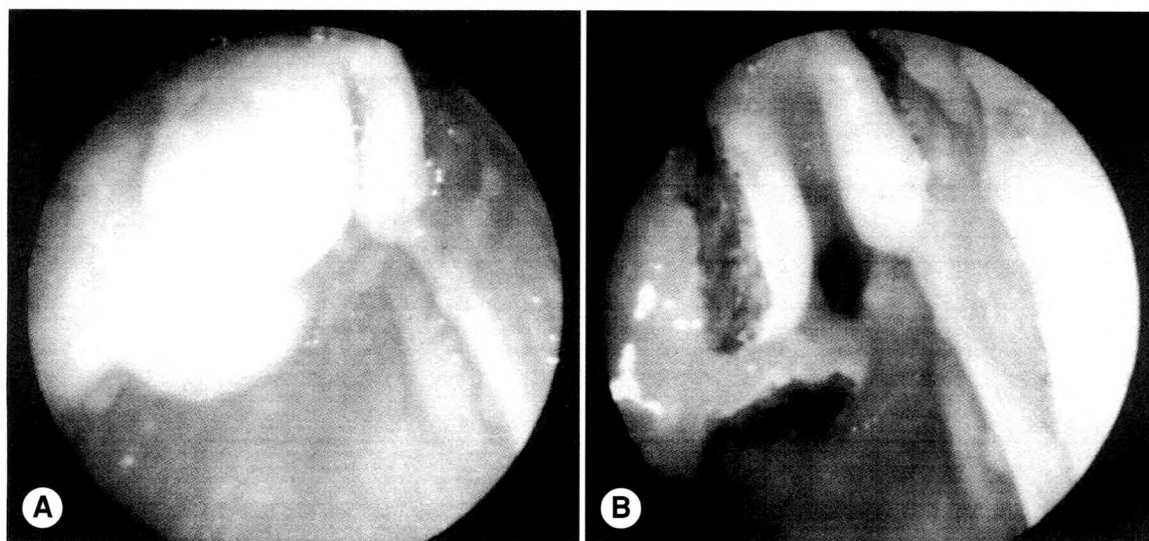


Fig 3. Lymphangioma involving epiglottis. Photographs taken **A)** before and **B)** after CO₂ laser excision.

as an effective and safe treatment for lymphangiomas.⁴² Real-time monitoring of tissue temperature allows controlled coagulation necrosis of targeted tissue, resulting in laser-induced fibrosis of the lymphatic tissue while preserving vital structures. Unfortunately, LITT is time-consuming and requires the availability of an open-configured MRI scanner. In addition, safety precautions must be followed in the use of the Nd:YAG laser.

Somnoplasty. Somnoplasty, or temperature-controlled radio frequency volumetric tissue reduction, is a minimally invasive surgical procedure. The literature on this new technique is sparse and limited to its use in snoring, mild sleep apnea, and turbinate hypertrophy. A needle radio frequency probe is used to ablate targeted tissue while leaving overlying mucosa and underlying structures intact.⁴³ Although its use has been described since 1999 in mucosally covered structures of the oral cavity and nasal chambers, reports on the external use of somnoplasty are not available.⁴⁴ At the University of Iowa, we have been delivering 1,000 J per treatment site to lymphatic malformations involving the oral cavity and base of the tongue, cheeks, and floor of the mouth. Thus far, we have not found any complications associated with the transmucosal use of somnoplasty.

Sclerosing Agents. The thought of treating lymphangiomas with sclerotherapy is not new. The idea emerged more than 50 years ago, when it was observed that cystic hygromas could shrink or regress completely after infection. Since then, many injectable agents have been used to imitate what may naturally occur.

Bleomycin sulfate, an antibiotic with antitumoral activity used as a chemotherapeutic agent, was proposed as a sclerosing agent for lymphangiomas in

1977 by Yura et al.⁴⁵ Although very good results have been reported, the potential risk of pulmonary fibrosis is an unacceptable one in the treatment of a benign disease.^{46,47}

Ethibloc is usually used in the treatment of venous malformations, but has been administered by percutaneous embolization in more than 100 patients with lymphangioma.^{48,49} Reported results have been very promising, with good or excellent responses in more than 60% of cases. The associated risks include inflammatory reactions and ulcerations. The Ethibloc also can extrude through the skin for weeks to months after injections, adversely affecting the final outcome.

Interferon alfa-2a has been effectively used in the treatment of hemangiomas in infants and children.⁵⁰ The occasional mixed nature of lymphangiomas (hemangiolymphangioma) prompted consideration of this treatment. Although results in a small cohort of patients have been favorable,⁵¹ we have not been able to document any benefit when interferon alfa-2a is used to treat lymphangiomas. Signs of toxicity are common and include fever, nausea, loose stools, neutropenia, alopecia, fatigue, and elevated liver enzyme levels.⁵¹

Since 1991, Castañón et al⁵² have been treating lymphangiomas with a fibrin sealant, Tissucol. The rationale behind the use of this hemostatic agent is its effectiveness in sealing tissue surfaces and eliminating potential dead spaces. In a recently published study,⁵² complete remission was demonstrated by US in 17 of 19 cases of lymphangiomas treated with aspiration and injection of Tissucol. The results are promising, and to date, no complications have been reported.

Over the past 14 years, numerous reports have de-

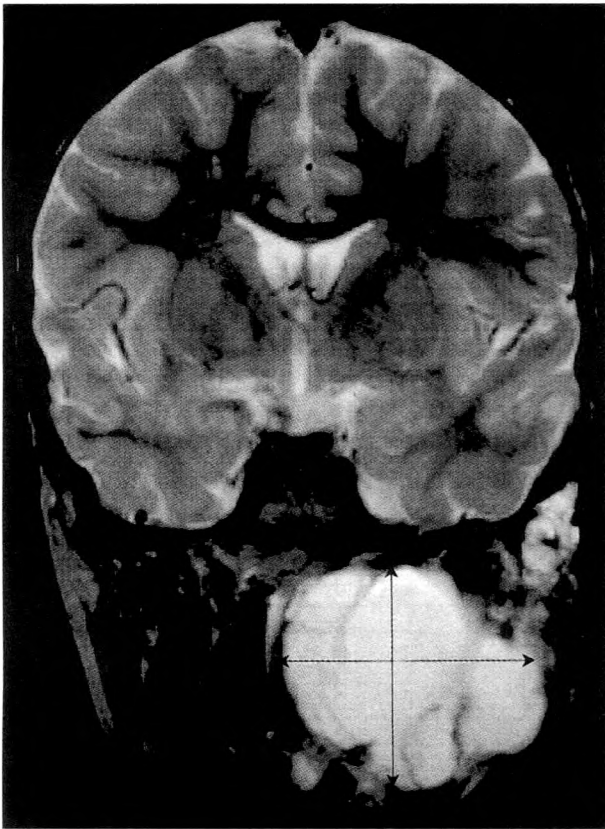


Fig 4. Measurements of large cystic hygroma taken before OK-432 sclerotherapy. Lesion volume is determined by product of orthogonal dimensions from magnetic resonance imaging scan.

scribed the successful use of OK-432 for the treatment of head and neck lymphangiomas in children.^{13-15,36,53-55} This potent immunostimulant is a lyophilized mixture of a low-virulence strain (Su) of group A *Streptococcus pyogenes* incubated with penicillin G.^{54,56-58} Known also as Picibanil, OK-432 causes immunologic up-regulation of several cytokines secondary to the antigenic stimulus induced by streptococci. It was developed initially for use in head and neck cancers, and although numerous clinical trials failed to demonstrate any antitumor effects, two important findings did emerge.⁵⁹ First, when OK-432 was injected into the pleural and peritoneal cavities to treat malignant effusions, it became apparent that it had sclerosant properties.⁶⁰ Second, in studies of more than 30,000 patients, no significant clinical toxicity was recorded.⁶¹

The sclerosing ability of OK-432 is related to its immunomodulatory activity, which has been studied in depth. OK-432 activates neutrophils, macrophages, natural killer cells, and T-cells. It also elevates many soluble immune mediators such as IL-1 (interleukin-1), IL-2, and NKAf (natural killer activating factor).⁶² Concentrations of both tumor necrosis factor and IL-6 increase in cyst fluid after in-

tracystic injection of OK-432.⁵⁷ Cellular stimulation of macrophages and lymphocytes precedes the increase in soluble immune mediators.⁶³

Prompted by the success of OK-432 as a sclerosing agent in malignant effusions, Ogita et al^{55,64} used OK-432 for large lymphangiomas and described their initial encouraging results in 1987 and 1991. In their most recent review, they reported that of 64 patients treated since 1986, more than 50% had experienced complete resolution of their disease, and in another 23% the response had been good (>50% reduction in size).⁵³ OK-432 was more effective as a primary therapeutic agent than as an adjuvant following surgical excision or bleomycin sclerotherapy. Their encouraging results suggested that carefully designed clinical studies should be conducted to examine the role of OK-432 as primary therapy for lymphangiomas in children.

We published promising results in 1996 and 1999 describing our experience with OK-432 in the treatment of lymphangiomas in children at the University of Iowa.^{13,14} Three years ago, we designed and began conducting a multi-institutional prospective randomized control study to assess the efficacy and safety of this therapeutic technique. Because OK-432 is currently not approved for wide-scale use in the United States, this trial was initiated as a first step toward the approval of this sclerosing agent by the US Food and Drug Administration.

The protocol and treatment procedures are relatively simple and easy to follow. In most circumstances, the treatments are performed under general anesthesia. A solution of OK-432 is prepared by dissolving 0.1 mg of OK-432 in 10 mL of normal saline solution. A 20-gauge angiocatheter needle is introduced into the cyst under sterile conditions. Once the needle is withdrawn, cyst contents are aspirated through the angiocatheter. Palpation, transillumination, US, and, in rare circumstances, fluoroscopy can aid in cyst localization. While ensuring that the catheter does not move after aspirating as much cyst fluid as possible, one injects the OK-432 solution. Single or multiple cysts can be injected, depending on the characteristics of the lymphangioma, to a maximal total dose of 20 mL in 1 treatment session. The injection sessions are spaced at 6 to 8 weeks for a total of 4 injection series, unless a contraindication arises or a complete response is observed. The outcome is determined by the reduction in lesion volume based on CT scan or MRI measurements obtained before starting treatment and after its completion. Clinical success is defined as a complete (>90%) or substantial (>60%) decrease in volume of the lymphangioma (Fig 4).

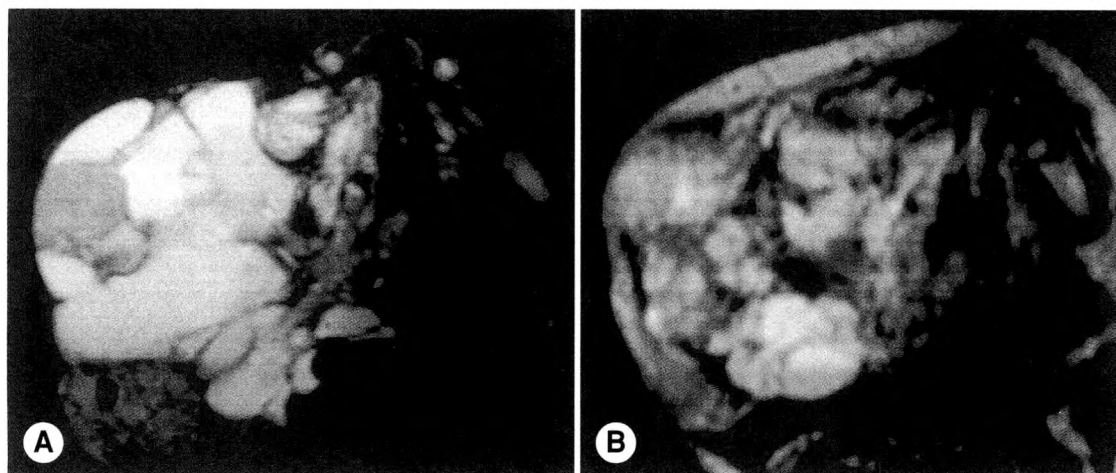


Fig 5. Magnetic resonance imaging scans of mixed lymphangioma involving right suprahyoid region A) prior to and B) after 4 OK-432 injection treatments, demonstrating no change in microcystic portion of lesion.

Our preliminary results are extremely encouraging. To date, our overall success rate of 66% is in keeping with other retrospective studies on the efficacy of OK-432 in the treatment of lymphangiomas.⁶⁵ When we exclude patients with microcystic disease, 86% of the patients had a successful response to therapy. Thus far, we find that macrocystic lesions respond significantly better than mixed or microcystic lesions — a finding that strongly supports previously published studies, which report the rate of complete response of macrocystic lesions to be 92% to 100%^{15,53} (Fig 5). Of our patients who were treated successfully, 95% had macrocystic lymphangiomas. The use of this agent in microcystic disease has failed to result in therapeutic benefits.

Like de Serres et al,¹⁶ we are finding that patients with stage I, II, or III disease show significantly greater response rates to OK-432 than do patients with stage IV or V disease. OK-432 sclerotherapy is prov-

ing to be very useful in lesions located in surgically challenging areas; all parapharyngeal lesions, as well as a case of a retropharyngeal lesion, have shown complete responses to treatment (Fig 6).

In contrast to other sclerosing agents, OK-432 has been administered routinely intradermally and intravenously in Japan without major side effects.⁶⁴ Therefore, one does not have to worry about accidental extralesional injection with this agent. So far, OK-432 treatment has not been reported to adversely affect subsequent surgical procedures. One report describes its use in utero for the treatment of a large cystic hygroma.⁶⁶ Further studies are necessary to evaluate the safety of intrauterine treatment with OK-432 sclerotherapy.

In general, the side effects are minor and short-lived. Erythema, swelling, discomfort at the injection site, and pyrexia are the most frequently reported, and typically resolve after the fourth posttreatment

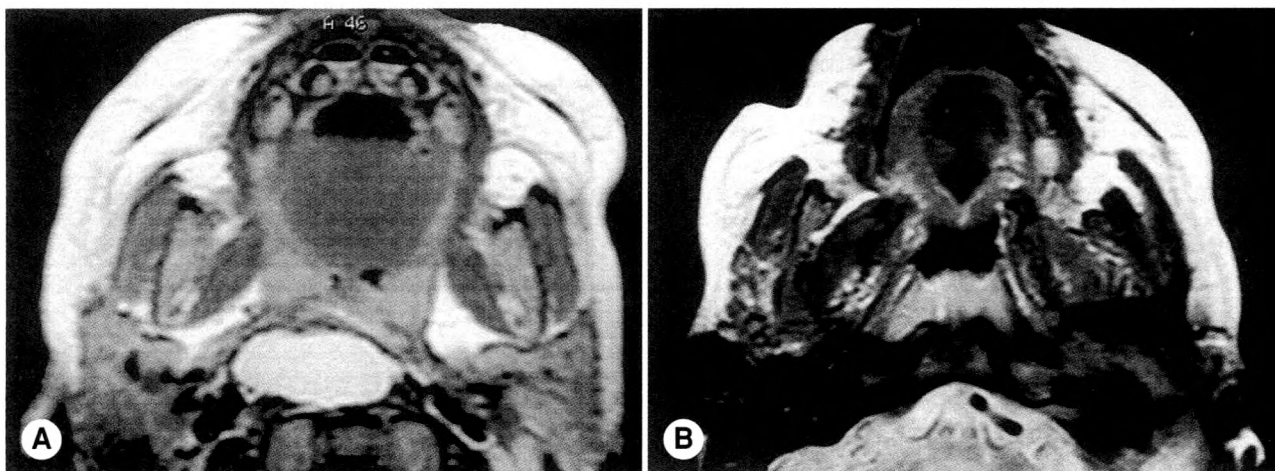


Fig 6. Magnetic resonance imaging scans. A) Large retropharyngeal lymphangioma causing upper airway obstruction. B) Complete resolution of lesion following 2 injection treatments of OK-432.

day. OK-432 should not be administered to patients who are allergic to penicillin because of the potential risk of anaphylactic shock.

Future Directions. The treatment of lymphangiomas is challenging. Although surgery remains an excellent therapeutic technique for microcystic and mixed lesions of the head and neck, many authors now recommend OK-432 sclerotherapy as primary therapy for macrocystic disease.⁵⁷ This therapeutic technique appears to be a relatively safe and effective treatment option for patients of all ages. The current ongoing multi-institutional trial should yield more results regarding its efficacy in the near future.

Research in the molecular biology of lymphangiomas is now a "hot topic." Understanding disease pathogenesis at the molecular level may generate new treatment options. A potential lymphatic endothelial growth factor receptor involved in developing lymphatic tissue, lymph nodes, and lymphangiomas has been discovered and may represent an opportunity to develop alternative treatment strategies for lymphatic malformations.⁶⁷

Vascular endothelial growth factor-C (VEGF-C), a ligand of VEGFR-3 and VEGFR-2, in the skin of transgenic mice results in lymphatic, but not vascular, endothelial proliferation and vessel enlargement. Because VEGF-C induces selective hyperplasia of lymphatic vasculature, it may play a role in disorders of the lymphatic system and may be of potential use in therapeutic lymphangiogenesis.⁶⁸ A study using a quail model has found similar and supporting results.⁶⁹ Control of lymphangioma growth potentially could be achieved by the development of an agent that would selectively block VEGF-C.⁷⁰

Another promising discovery is the finding of high levels of basic fibroblast growth factor, an angiogenic inducer, and low levels of thrombospondin-1, a naturally occurring angiogenic inhibitor, in isolated cystic hygroma cells.⁷⁰ This observation may mean that lymphangiomas are dependent on excessive angiogenesis for growth and development. If this hypothesis is correct, the value and efficacy of antiangiogenic therapy in suppressing the growth of these lesions can be tested.

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REPAIR OF A TYPE IV LARYNGOTRACHEOESOPHAGEAL CLEFT WITH CARDIOPULMONARY BYPASS

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Laryngotracheoesophageal clefts (LTECs) are rare congenital defects of variable severity depending on the extent of malformation. Management of a complete LTEC represents a major surgical and anesthetic challenge. The main problems are achieving adequate operative exposure and maintaining ventilatory support during and after the operation. We describe correction of a type IV LTEC extending down to the carina in an infant who had respiratory distress at birth. Surgical repair was achieved in a single stage by an anterior sternotomy approach on the 11th day of life. The procedure was facilitated by cardiopulmonary bypass. After the operation, the infant was intubated, mechanically ventilated, and sedated. Nissen fundoplication and gastrostomy were carried out on the 11th postoperative day. The child was extubated on the 12th postoperative day. The rationale for this method and its advantages in comparison with other surgical approaches are discussed.

KEY WORDS — cardiopulmonary bypass, congenital laryngotracheoesophageal cleft, difficult pediatric airway, surgical repair.

INTRODUCTION

Laryngotracheoesophageal clefts (LTECs) are rare congenital defects of the aerodigestive tract caused by anomalies that occur during the generation of supporting tracheal cartilage and the fusion of the tracheoesophageal folds.^{1,2} The presenting symptoms include stridor, excessive secretions, choking, cyanosis, and aspiration into the lungs after feeding. The diagnosis is confirmed by endoscopic examination. Various classifications have been proposed³⁻⁶ based on anatomic and clinical findings. The Benjamin and Inglis⁶ classification includes 4 categories of clefts. Type I is a supraglottic interarytenoid cleft located above the vocal cords. Type II is a partial cricoid cleft extending below the vocal cords. Type III is a total cricoid cleft extending through the cricoid cartilage with or without further extension into part of the cervical tracheoesophageal wall. Type IV is a total cricoid cleft with major or total involvement of the thoracic tracheoesophageal wall.

Early surgical repair is mandatory to prevent life-threatening pulmonary complications due to persistent aspiration of stomach contents.⁷ The high mortality rate is related to the magnitude of the cleft, concurrent congenital anomalies, delay to effective repair, and difficulty in maintaining a stable airway.^{8,9} This report describes a case of type IV LTEC extending to the carina that was successfully repaired by an anterior laryngotracheal approach facilitated by cardiopulmonary bypass (CPB).

CASE REPORT

The patient was a 2,360-g girl born at 38 weeks' gestation to a 25-year-old gravida 2 para 1 mother. The pregnancy was complicated by arterial hypertension treated by methyldopa and nicardipine hydrochloride during the third trimester. Labor was induced at an outside hospital because of the mother's uncontrolled hypertensive crisis. The Apgar score was 6 at 1 minute and 10 at 5 minutes with spontaneous crying. Respiratory distress was noted shortly after delivery, and the infant was transferred to a neonatal intensive care unit, where continuous positive airway pressure (CPAP) was instituted with 30% FiO₂ (fraction of inspired oxygen). Initial chest x-ray findings appeared normal, but subsequent views revealed distention of the stomach. A diagnosis of LTEC was made on the 4th day of life during laryngoscopy and tracheobronchoscopy by an experienced pneumologist.

On the 5th day of life, the infant was sent to the pediatric intensive care unit of La Timone Children's Hospital for further evaluation and treatment. The SpO₂ (oxygen saturation as measured by pulse oximetry) remained between 94% and 99% with the child breathing 0.5 L/min of oxygen by nasal prongs. The PaCO₂ (partial pressure of carbon dioxide, arterial) was between 65 and 77 mm Hg. Laryngoscopy, tracheobronchoscopy, and esophagoscopy performed under general anesthesia with spontaneous breathing demonstrated a type IV LTEC extending from

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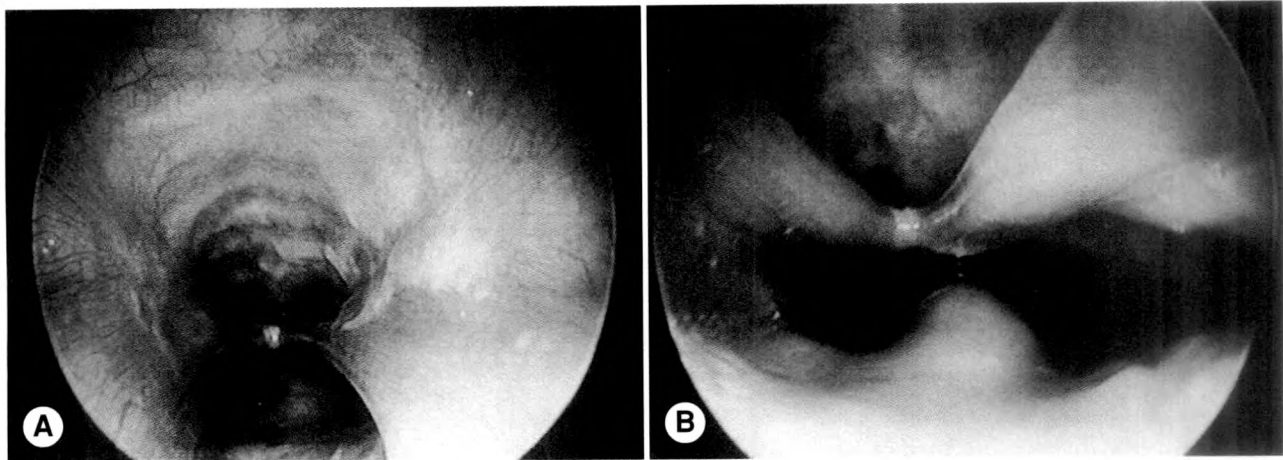


Fig 1. Endoscopic views. **A)** From airway side, documenting laryngotracheoesophageal cleft extending down to carina. **B)** From esophageal side, showing similar findings.

the interarytenoid area down to the carina without accompanying esophageal atresia (Fig 1).

Over the ensuing days, the patient was kept on parenteral nutrition with nothing given by mouth. Continuous aspiration with an oropharyngeal tube was performed to prevent accumulation of oral secretions. Intravenous omeprazole (6.6 mg/d) was administered to reduce the risk of gastroesophageal reflux and aspiration pneumonia. The patient's hemodynamic status remained stable with an SpO₂ of more than 94% with nasal oxygen delivery. Physical examination, a complete skeletal x-ray scan, and abdominal, cardiac, and brain ultrasound scans showed no evidence of associated malformation. Surgical correction was undertaken on the 11th day of life, after informed consent was obtained from the parents.

After oxygenation, anesthesia was induced with sevoflurane in 100% oxygen. To secure the airway, we inserted two 2.0-mm-internal diameter endotracheal tubes, one in each main stem bronchus. The infant was maintained in the supine position with the

head extended. The position of the tubes was checked with a 4-mm direct endoscope. Once it was assured that both lungs were being ventilated, the two tubes were secured and the endoscope was withdrawn. The two tubes were connected by a double T-piece, and mechanical ventilation was performed via a Siemens Servo D ventilator. Anesthesia was maintained with a combination of intravenous agents including sufentanil citrate, midazolam, and atracurium besylate.

The first step in the procedure involved preparation for CPB by the cardiovascular team. After median sternotomy, cannulae were placed in the ascending aorta and right atrium. Care was taken to position the cannulae so that they would not interfere with operative exposure. Next, the anterior laryngotracheal approach was extended to allow division of the trachea, cricoid cartilage, and lower thyroid cartilage. At this point, CPB was initiated and the two endotracheal tubes were removed, thus maximizing exposure of the cleft. A tracheoesophageal septum was created from mucosal flaps dissected from the lateral shelves of the cleft (Fig 2). Two-layer closure

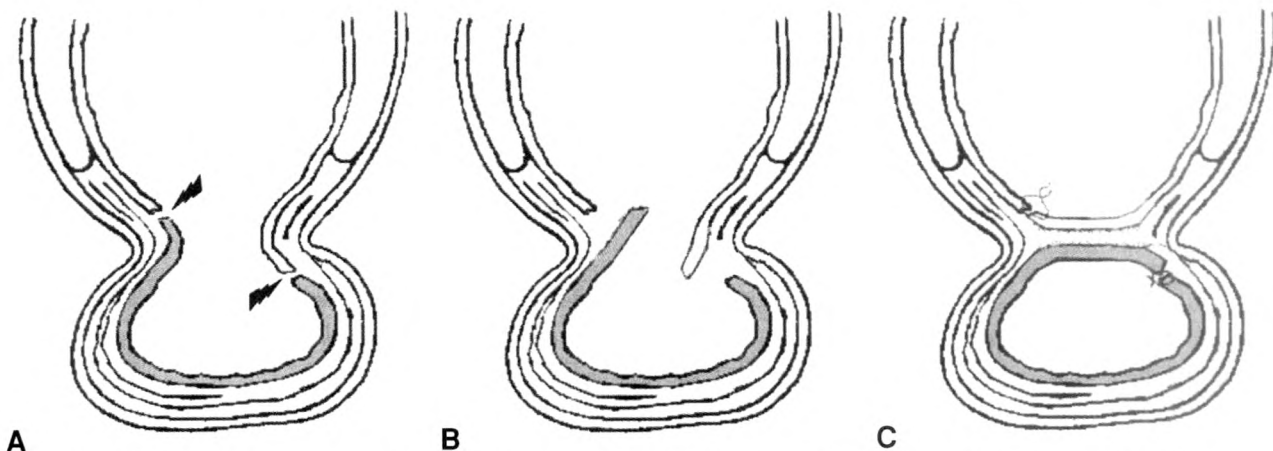


Fig 2. **A,B)** Development of mucosal flaps from transesophageal "shelves." **C)** Two-layer closure of tracheoesophageal septum with nonoverlapping suture lines.

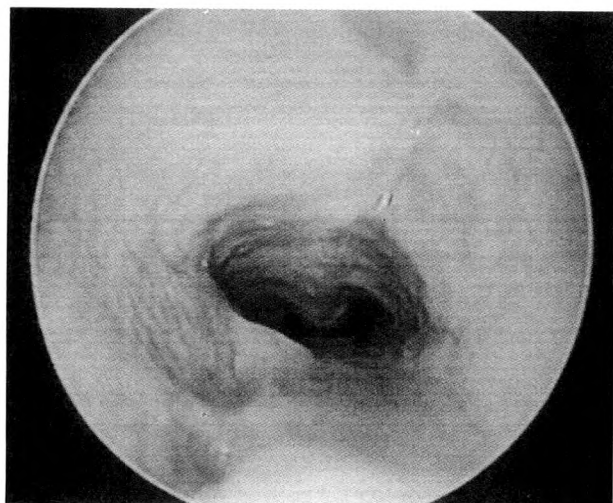


Fig 3. Endoscopic view from airway side shows adequate healing of suture line at postoperative month 1.

using 5.0-polydioxanone suture was effected with nonoverlapping sutures. Before complete closure of the anterior wall of the trachea, a 3.0-mm-internal diameter nasotracheal tube was introduced and CPB was stopped. The total duration of CPB was 138 minutes. The surgical wound was closed over a suction drain. The total duration of anesthesia was 10 hours. At the end of the procedure, the patient was transferred to the pediatric intensive care unit. The thoracic drain was removed 48 hours after the operation; only 40 mL of blood was collected through the drainage tube.

Two complications occurred during the postoperative period, ie, low cardiac output and acute respiratory failure. The low cardiac output could have resulted from myocardial dysfunction related to intraoperative support technique (effects of CPB) in a newborn. It was successfully treated by continuous intravenous infusion, first of dopamine chlorhydrate ($10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and then of epinephrine ($0.25 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Peritoneal dialysis was instituted because of marked oliguria in spite of improving arterial pressure and the continuous infusion of furosemide. The acute respiratory failure might have been related to frequent aspiration during the preoperative period. The patient was treated by mechanical ventilation with 100% oxygen and high positive end expiratory pressure (PEEP; 6 to 10 mm Hg) for the first 24 hours to prevent hypoxia (PaO_2 [partial pressure of oxygen, arterial], 54 to 78 mm Hg). The patient was kept sedated and relaxed by continuous intravenous infusion of fentanyl citrate, midazolam, and atracurium. At the end of 1 week, the controlled mechanical ventilation was discontinued and intermittent ventilation was instituted. The ventilation assistance was gradually decreased afterward, but the nasotracheal tube was kept in place as a stent. After 2

episodes of emesis on the 10th postoperative day, Nissen fundoplication and gastrostomy were carried out on the 11th postoperative day. Since concurrent laryngoscopy, tracheobronchoscopy, and esophagoscopy demonstrated sufficient cicatrization of the tracheoesophageal septum, the endotracheal tube was removed on the 12th postoperative day. No further aspiration was observed, and the SpO_2 remained adequate without supplemental oxygen.

On the 15th postoperative day, total parenteral nutrition was stopped and enteral feeding through the gastrostomy was started. Endoscopy at 1 month demonstrated that the suture line was intact on both the tracheal and esophageal sides of the septum (Fig 3). Oral feeding was attempted on the 30th postoperative day, but could not be continued because of aspiration. An upper gastrointestinal series showed no evidence of fistula and suggested that the aspiration was due to decreased pharyngoesophageal motility. The infant was discharged from the hospital on the 35th postoperative day. After 2 months of physiotherapy to improve pharyngoesophageal function, another upper gastrointestinal series was performed, showing no sign of aspiration. By the end of the 3rd postoperative month, oral feeding was adequate and the gastrostomy tube was removed. The child is currently 32 months old and has normal voice and oral food intake.

DISCUSSION

Successful treatment of a type IV LTEC depends on maintaining adequate ventilation and preventing gastroesophageal reflux (GER) and aspiration in the preoperative period.¹⁰ In our case, adequate oxygenation was maintained by spontaneous breathing with oxygen delivery through nasal cannulae. Continuous nasopharyngeal suctioning was performed to remove oral secretions. To minimize GER, we maintained the patient in an upright position and administered omeprazole. Feeding was performed by the parenteral route.

The decision to perform operative repair depends on the overall status of the patient. Two important prognostic factors are the magnitude of the LTEC and coexistence of other congenital anomalies. In an 85-case review, Roth et al⁸ reported that the mortality rate ranged from around 43% for types I, II, and III to 90% for type IV. Concurrent congenital malformations are found in 56% of patients with LTECs and in 67% of fatal cases.¹¹ Tracheoesophageal fistula has been reported in 20% to 27% of type I, II, and III LTECs and may cause a poor outcome if not corrected.¹¹⁻¹³ Laryngotracheoesophageal clefts have also been reported in association with Opitz syndrome and Pallister Hall syndrome.^{14,15}

A major problem in type IV LTEC repair is achieving adequate exposure. As have many other clinicians,^{2,5,11} we preferred the anterior laryngotracheal approach with median sternotomy to lateral pharyngotomy with right thoracotomy.^{7,9} The main advantages of the anterior approach are to provide excellent direct operative access with minimal dissection at the cervical level and no risk of recurrent nerve damage.

Another critical challenge in LTEC repair involves maintaining adequate ventilation of both lungs during the procedure. Ryan et al⁹ and Donahoe and Gee¹⁶ used custom-made bifurcated endotracheal tubes to achieve bilateral ventilation. Since these tubes were not available at our institution, we used two 2.0-mm-internal diameter endotracheal tubes, one placed separately in each main stem bronchus. The two tubes were connected by means of a double T-piece. In this way, it was possible to perform ventilation with a single ventilator. However, the presence of the endotracheal tubes hinders access to the surgical site during the repair phase.

Two solutions could have been used to obviate the need for cumbersome endotracheal tubes and maximize exposure during repair, ie, CPB and extracorporeal membrane oxygenation (ECMO). We opted for CPB because of its availability and relative simplicity. The main drawback to CPB is that it cannot be applied for longer than several hours. This problem was resolved by limiting the use of CPB to the repair phase of the procedure so that the duration of bypass was only 138 minutes. However, the duration of anesthesia was much longer, because this case was very complex. A good portion of the anesthesia time was spent ensuring that the endotracheal tube placement and ventilation were adequate before commencing with surgical incision, and dealing with low cardiac output and respiratory failure in the early postoperative period. Possible advantages of ECMO would have been to allow ventilatory support for a period of several days and to avoid postoperative trauma.¹⁷ Use in our patient might have avoided the

two postoperative complications, ie, low cardiac output and acute respiratory failure. However, ECMO is a demanding technique that requires repeated repositioning of the cannulae and/or adjustment of the circuitry both during and after the operation. Another drawback of ECMO is the risk of devastating central nervous system hemorrhage.

Protection of fresh sutures is an important issue for LTEC repair. Two important precautions against fistulous complications and cleft recurrence are 2-layer closure with nonoverlapping sutures^{2,11,16} and single-stage repair whenever possible.⁸ Postoperative suture-related complications have also been correlated with GER and nasogastric tube placement. According to Hof et al,¹⁸ clinically significant GER can cause suture breakdown for up to several weeks after successful LTEC repair. Nasogastric tubes can result in pressure necrosis. To avoid these problems, many authors recommend Nissen fundoplication and gastrostomy prior to cleft repair. In our patient, 2 episodes of GER occurred on the 10th postoperative day, and Nissen fundoplication with gastrostomy was carried out the next day.

Successful repair of an LTEC may not lead to immediate restoration of normal function. In most cases, oral feeding must be started progressively to allow establishment of the swallowing reflex and pharyngoesophageal motility.^{2,11} In our patient, normal oral feeding was achieved 2 months after the operation, after physiotherapy to improve pharyngoesophageal motility.

CONCLUSION

This report describes a case involving 1-stage correction of a type IV LTEC using CPB. This technique facilitated the repair phase of the procedure by ensuring adequate blood oxygenation and maximizing operative exposure. Use of CPB should be considered in any situation in which endotracheal intubation may interfere with operative exposure. In the postoperative period, care must be taken to prevent suture line damage due to positive pressure ventilation and GER.

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NASAL POLYPOSIS IN CHILDREN WITH CYSTIC FIBROSIS: A LONG-TERM FOLLOW-UP STUDY

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The incidence of nasal polyposis among children 5 to 18 years of age with cystic fibrosis (CF) was investigated with a systematic examination of all children on the local CF register. Out of 23 children with CF, 13 had endoscopic evidence of nasal polyposis. Four children had grade 2 polyposis, and 9 children had grade 3 polyposis. Complete opacity of the maxillary sinus was identified on a computed tomographic sinus scan in all but 2 of the children. Only 1 child had a developed frontal sinus. Between 1989 and 2000, 12 children underwent radical endoscopic sinus surgery for their nasal polyposis. There was good postoperative improvement in all of the children; however, 7 eventually required revision surgery because of recurrence of the nasal polyps. The median interval between repeated sinus surgeries was 4 years (range, 18 months to more than 6 years). This information can help in the counseling of parents when sinus surgery is considered for children with CF.

KEY WORDS — cystic fibrosis, nasal polyposis, sinus surgery.

INTRODUCTION

Cystic fibrosis (CF) is a genetic disease with autosomal recessive inheritance. The CF gene is located at chromosome 7 and is responsible for the CF transmembrane regulator (CFTR). The CFTR is a membrane-bound protein that has been shown to be capable of chloride ion transport.¹ Deficiency of CFTR in a CF sufferer causes chloride channel dysfunction at the epithelial cells lining the airway and the exocrine glands. It results in a flux of sodium chloride into the epithelial cells, leading to dehydration of the extracellular fluids and accumulation of thickened inspissated mucus secretions in the air passage and the sinus cavity.² Hence, chronic rhinosinusitis can be one of the manifestations of CF in children. In rare circumstances, sinonasal disease may even be the initial presenting symptom in CF.³

The overriding concerns in children with CF are usually recurrent or chronic chest infections and malabsorption due to pancreatic insufficiency. It is relatively unusual for otolaryngologists to be involved in the routine management of children with CF. In most cases, the patients are referred to otolaryngologists only if the child develops nasal symptoms. Since 1989, the senior author (M.W.Y.) has provided a systematic rhinologic assessment of all children with CF at the Ipswich Hospital National Health Service (NHS) Trust, which serves a population of 350,000. The local population in East Suffolk tends to be rela-

tively settled; hence, it allows the senior author to follow up these children for long periods.

PATIENTS AND METHODS

Since 1989, the senior author has been providing a routine assessment of all of the children with CF in East Suffolk. As part of the initial assessment by the pediatricians at the Ipswich Hospital NHS Trust, nasal symptoms were already systematically inquired after and recorded on the clinical records of all children with CF. These children were identified from the local CF patient register, and they were called for a full otorhinolaryngological examination by the senior author once they reached the age of 5 years. Also as part of the routine CF assessment, all of the patients had regular sputum and cough swab culture. Nasal endoscopy was performed in all patients. Children who did not have nasal symptoms or endoscopic evidence of nasal polyposis were followed up at the ear, nose, and throat (ENT) clinic only once every year. As part of the routine sinus assessment, a computed tomographic (CT) scan of the sinuses was requested on all children with CF. Occasionally, children were referred to the ENT clinic at the request of a pediatrician even before 5 years of age, but many were not yet cooperative enough for rigid nasal endoscopy or a CT scan of the sinuses. These procedures were delayed until the child became more cooperative at an older age.

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TABLE 1. MEAN CT SCORE (ACCORDING TO LUND-MACKAY STAGING) OF EACH SINUS AREA IN CHILDREN WITH CYSTIC FIBROSIS

Sinus Area	Children With Sinus Surgery (n = 12)		Children Without Sinus Surgery (n = 11)	
	Right	Left	Right	Left
Maxillary sinus	1.92	2	1.55	1.64
Anterior ethmoid sinus	1.75	1.83	0.64	0.73
Posterior ethmoid sinus	1.67	1.58	0.55	0.45
Sphenoid sinus	1.45	1.63	1.00	1.00
Frontal sinus	*	*	2.0†	2.00†
Ostiomeatal complex	2	2	1.64	1.64

Maximal score of each sinus site on one side is 2.

CT — computed tomography.

*Frontal sinus in all children was hypoplastic.

†Only 1 of 11 children had developed frontal sinus, which was still small.

It is the philosophy of the authors that improvement of the quality of life is an important aspect of the management of children with CF, and therefore chronic rhinosinusitis was treated vigorously. Many of these children were already on long-term prophylactic antibiotic treatment for recurrent chest infections. Some also received nebulized colistin for *Pseudomonas* chest infection. Children with early nasal polyposis were treated routinely with steroid nasal drops. However, topical antibiotics were not used as treatment for nasal polyposis in these children. The decision for sinus surgery was made jointly by the otolaryngologist (M.W.Y.) and the pediatrician (J.G.). Children with severe nasal symptoms associated with nasal polyposis were offered surgery. For those children who required surgery, the parents were warned that repeated surgery was inevitable. The children were admitted several days before the operation under the care of the CF pediatrician (J.G.) for cardiopulmonary workup in preparation for the operation. Because all of the children invariably had extensive nasal polyposis and pansinusitis, they were treated with radical endoscopic sinus surgery including total ethmoidectomy, middle meatal antrostomy, and sphenoidotomy. The anterior and inferior portions of the middle turbinate were resected. The end result was a cavity in front of the anterior skull base widely communicating with the large sinuses. The authors did not perform routine coagulation screening before 1990. Since 1990, all children 5 years of age and older have had a yearly prothrombin screening and liver function test as part of the workup. None of the patients showed any evidence of coagulation defect. Indeed, troublesome bleeding was not encountered in any children with CF during the endoscopic operation, even though the middle turbinates were partially resected in all cases. The endoscopic sinus surgery

TABLE 2. SUMMARIZED FREQUENCY DISTRIBUTIONS OF CT SCORES FOR CHILDREN WITH AND WITHOUT SURGERY

	Total Score				
	0	1-9	10-14	15-19	20
Surgery	0	0	3	3	6
No surgery	2	0	7	2	0

Data are totaled over 10 regions for which full data were available.

and nasal polypectomy were carried out with endoscopic surgical instruments rather than a microdebrider. All of the patients received antibiotics during and after operation, but systemic steroids were not routinely given before or after the operation. After the sinus operation, the children were instructed to perform douching of the nose with saline solution and to use steroid nose drops daily on a long-term basis. Topical antibiotics such as gentamicin lavage were not used as follow-up treatment, although this has been suggested by other workers.⁴ Once the sinus cavity healed, the patient was followed up at the ENT clinic every 6 months for assessment of progress. None of the patients were discharged from the clinic, in order to maintain long-term follow-up. The patients, or their parents, were asked on each attendance to rate the symptoms as being worse, the same, or better since the operation. Specific nasal symptoms were evaluated, including nasal obstruction, nasal discharge, sense of smell, postnasal discharge, facial pain, and headache. Nasal endoscopy was performed routinely on each visit, and any recurrence of nasal polyposis was recorded.

RESULTS

Between 1989 and 2000, 34 children (initially 18 years of age or younger) were on the CF patient register at East Suffolk. Eleven children were younger than 5 years of age, and therefore only 23 of a total of 34 children with CF were called for a full otorhinolaryngological assessment including a CT scan of the sinuses. The results of the CT scan in these 23 children, based on the Lund-Mackay CT staging or scoring system,⁵ are summarized in Table 1. Complete opacity of the maxillary sinus was identified in all but 2 of the children examined. A very common feature was the demineralization of the uncinate process and medial displacement of the lateral nasal wall in the middle meatus, which was observed in three quarters of the patients. Another typical CT finding in children with CF was hypoplasia of the frontal sinus. A summary of the total CT scores over 10 of the 12 regions (omitting the frontal sinus, for which scores were available for just 1 patient) is given in Table 2. As expected, the group of children who were offered sinus surgery had significantly higher total CT scores ($\chi^2 = 10.2$; $p < .01$) than did the rest of the children.

TABLE 3. INDICATIONS FOR SURGERY AND SELF-RATING OF CHANGE OF SYMPTOMS OF CHRONIC SINUSITIS SIX MONTHS AFTER RADICAL ENDOSCOPIC SINUS SURGERY

Patient No.	Indications for Surgery	Nasal Blockage	Nasal Discharge	Anosmia	Postnasal Drip	Facial Pain or Headache
1	PO	Better	NS	NS	NS	NS
2	TO, An	Better	NS	Better	NS	NS
3	TO, An, ND, PND	Better	Better	Better	Better	NS
4	TO, An, PND	Better	NS	Better	Better	NS
5	TO, An, ND	Better	Better	Better	NS	NS
6	TO, An, ND, PND	Better	Better	Better	Better	NS
7	TO, An, ND, PND	Better	NS	Better	NS	NS
8	PO, An, PND	Better	NS	Better	Better	NS
9	TO, An	Better	Better	Better	Same	NS
10	TO, An	Better	NS	Better	Better	NS
11	PO, PND	Better	NS	NS	Better	NS
12	TO, An	Better	NS	Better	NS	NS

PO — partial nasal obstruction, NS — absence of symptoms both before and after operation, TO — total nasal obstruction, An — anosmia, ND — nasal discharge, PND — postnasal discharge.

Of the 23 children with CF assessed by the senior author, 13 had evidence of nasal polyposis. According to the staging system for nasal polyps recommended by Mackay and Lund,⁶ 4 patients had grade 2 polyposis and 9 patients had grade 3 polyposis. Endoscopic sinus surgery was performed in all of the children who had grade 3 nasal polyposis and in 3 of the 4 children who had grade 2 polyposis. On top of that, another child had submucous diathermy to the enlarged inferior turbinates as a result of allergic rhinitis, but that was not associated with nasal polyposis. Of those children who underwent endoscopic sinus surgery, 7 were male and 5 were female. There was no clear relationship between CF genotype and the severity of polyposis. Seven of the 12 who underwent operation were homozygous for delta F508, and the other 5 were compound heterozygotes for delta F508 and another gene. Of the remaining 11 patients who did not undergo operation (age 5 to 18 years), 5 were homozygous for delta F508, no data were available on 2 patients, and 4 were compound heterozygotes for delta F508 and another gene. The median age of the children when they had their first sinus operation was 8 years (range, 5 to 16 years). Although the number of children in the present study was small, it does show a trend of maximum disease activity in the nose between 8 and 10 years of age, as 7 of the 12 children who had sinus operations were in this age group at the time of their first operation. Two of the children who had sinus surgery were twins. The majority of those who underwent surgery (7 of 12 patients) had *Pseudomonas aeruginosa* colonization of upper or lower airways on sputum or cough swab culture before operation. In 1 child, *Haemophilus influenzae* was cultured, and in the other 4 children, only commensal organisms or no organisms were identified. One surprising observation was that

none of the 23 children had chronic serous otitis media, and hence none of the patients required grommet insertion. The indications for surgery for each patient are shown in Table 3. Nine of the 12 children with endoscopic sinus surgery had complete nasal obstruction and total anosmia due to grade 3 nasal polyposis. Three children had partial nasal obstruction from the nasal polyps, but also symptoms of chronic sinusitis that were affecting their quality of life.

The operative findings for all of the children were very similar. Extensive polyps were found in the nose and almost all of the sinuses. Pyocele of the ethmoid and maxillary sinuses with thick, green inspissated pus was found in all of the patients. In 10 of the 12 children, the sphenoid sinus was also filled with inspissated pus. In spite of extensive surgery on the sinuses, there was no perioperative or postoperative complication in any of the patients. There were no cardiopulmonary complications, and none of the patients stayed in the hospital for more than 2 days after surgery.

Specific symptoms of chronic sinusitis were systematically inquired after on all of the follow-up visits. The children or their parents were simply asked to rate the various symptoms as worse, better, or the same after the operation. A more sophisticated assessment, such as a symptom severity score, was thought to be impractical for some of the young children in the present study. The symptomatic improvements reported by the parents 6 months after the first sinus operation are shown in Table 3. None of the children complained of sinus pain either before or after the operation. In fact, pyocele of the paranasal sinuses in these children did not seem to give rise to headache or sinus pain. The improvements enjoyed by almost all of the patients were unblocking of the nose and

TABLE 4. PROGRESS OF CHILDREN WITH CYSTIC FIBROSIS AFTER RADICAL ENDOSCOPIC SINUS SURGERY

Patient No.	Age at First Operation (y)	Interval Between 1st and 2nd Operations (y)	Interval Between 2nd and 3rd Operations (y)	Interval Between 3rd and 4th Operations (y)
1	5	1½*		
2	8	1*		
3	8	2*		
4	7	2½	1*	
5	5	1½	2½*	
6	15	4	1½*	
7	8	5½	1*	
8	16	4½	2½*	
9	8	4	6*	
10	10	3	2	6½*
11	9	1*		
12	9	1*		

*No further operation yet required or planned.

improved olfactory ability. In every patient, the sinus cavity healed with a polypoid mucosal lining. Eventually, nasal polyps recurred in all patients, although most patients remained relatively free of symptoms for a few years. Between 1989 and 2000, 7 children underwent revision sinus surgery because of a return of nasal symptoms. Table 4 illustrates the progress of each patient and the intervals between repeated sinus surgeries. One patient had 3 revision operations for recurrent nasal polyps. The shortest interval between repeated operations on the sinuses was 18 months, and the longest interval was more than 6 years. The median interval was 4 years.

DISCUSSION

The diagnosis of chronic rhinosinusitis in children with CF is not always easy. Headache or facial pain is extremely uncommon in children with CF.⁷ Most parents or patients reported symptoms of nasal blockage, purulent rhinorrhea, and anosmia.^{7,8} In a child, it may be sometimes difficult to differentiate between the symptoms of chronic rhinosinusitis, adenoid hypertrophy, and allergic rhinitis. On the other hand, the diagnosis of nasal polyposis is much more straightforward, provided the child can cooperate with the procedure of nasal endoscopic examination. In the present study, 13 of 23 children with CF (57%) between 5 and 18 years of age had nasal polyposis, and 9 of them had total nasal obstruction. The Ipswich Hospital NHS Trust is a district general hospital serving a population of 350,000, rather than a tertiary referral center; therefore, this figure represents an undistorted incidence of nasal polyposis among children with CF between the ages of 5 and 18 years. A previous report from Texas Children's Hospital⁹ re-

vealed that 22 of 93 patients with CF between 5 months and 27 years of age had evidence of nasal polyposis based on anterior rhinoscopy. The higher incidence of nasal polyposis in our study may be a result of the selected age group. Also, the use of a rigid nasal endoscope allows the detection of even small polyps within the middle meatus of the nose.

Up to the present, there were not many reports in the literature of systematic CT scanning of the sinuses in children with CF. April et al¹⁰ studied the CT scans of 12 children with CF who had symptoms of rhinosinusitis and observed some common features, including hypoplasia of the frontal sinus, total opacity of the maxillary sinus with medial displacement of the lateral nasal wall, and uncinate process demineralization.

The present study included all children with CF between 5 and 18 years of age, with or without symptoms of rhinosinusitis. It is surprising that 2 children in the present study had relatively normal-looking sinus scans (total CT scores of less than 4). Neely et al⁹ also investigated 93 patients with CF between the ages of 5 months and 27 years with radiography of the sinuses and reported that the developed sinuses were opaque in every patient.

The treatment of nasal polyposis in children with CF often depends on the philosophy and the enthusiasm of the surgeon. It has to be recognized that these children represent an increased anesthetic risk even for a simple polypectomy operation. Furthermore, the mucosal disease can never be eradicated by surgery. Some clinicians advocate extensive sinus surgery^{11,12} for nasal polyposis, whereas others advocate simple nasal polypectomy in order to shorten the anesthetic time.^{13,14} In recent years, endoscopic sinus surgery has been used by many surgeons, although the extent of the surgery still varies according to the enthusiasm of the surgeons.^{15,16} Many published reports were from tertiary referral centers,^{11,13} and hence the follow-up studies were reviews of patients over relatively short periods rather than long-term longitudinal studies.

In general, we advocate the use of intranasal corticosteroids for patients with CF who have grade 1 or even grade 2 nasal polyposis. Surgery is recommended for those with significant nasal symptoms that do not respond to conservative treatment. Accepting that recurrence of the nasal polyps is inevitable, the senior author prefers a radical sinus operation via an endoscopic approach for children with CF. As a matter of routine, total ethmoidectomy is performed in all of the patients. The maxillary and sphenoid sinuses are widely opened into the nasal cavity. Fortunately, the frontal sinus is usually poorly developed

in children with CF, and therefore surgery on the frontal sinuses is often not necessary. Routine trimming of the middle turbinate allows douching of the nose and the communicating sinuses following the operation to be more effective. A theoretical benefit of regular nasal douching is to mechanically remove mucus collected in the nose and sinuses to prevent secondary infection. Of a total of 23 children with CF between 5 and 18 years of age assessed by the authors over a 12-year period, 12 (52%) have undergone endoscopic nasal operation. This proportion does seem to be high compared to other series. Brihaye et al¹⁷ reported on a series of 248 patients with CF, of whom only 55 underwent endoscopic operation. This difference is most probably a result of the different cohorts of patients reported. Our study is confined to an age group of 5 to 18 years and is based on a longitudinal study of a stable population over time. Many children with CF underwent nasal surgery 5 to 10 years after the initial presentation to the authors. The children who underwent surgery eventually had symptoms that had compromised their quality of life. All of the patients had nasal obstruction and symptoms of chronic sinusitis such as anosmia and anterior or posterior nasal discharge (Table 3). Thus, the indications for surgery as reported in the present study did not differ from those of other reports.^{4,17,18}

It is the authors' opinion that the reporting of the outcome following sinus surgery in children with CF should not be based on the recurrence rate of the nasal polyps, as recurrence is regarded as inevitable if the patient is followed up for long enough. Instead, the results should include the average interval between revision surgeries. Drake-Lee and Morgan¹³ reported

that the average time between recurrences of the nasal polyps following simple polypectomy was 18 months, whereas the median time between revision surgeries in the present study was 4 years. It thus appears that radical sinus surgery offered a longer remission period than simple polypectomy. A similar conclusion has been reported elsewhere.¹¹ However, up to now, there has not been a single randomized study in the literature comparing the long-term results between simple nasal polypectomy and radical sinus surgery on children with CF. The senior author attempted to conduct such a study in 1989. It was abandoned because pyocele of the maxillary sinus was identified in all of the children with CF at the time of surgery, and it would have been unethical to leave the pyocele behind in half of the children.

The senior author has performed more than 2,000 endoscopic sinus operations. It must be said that the most satisfying ones have been in children with CF. These children have a reduced life expectancy that makes even a few years of symptomatic relief very worthwhile. The benefits of the operation, such as the relief of nasal obstruction and reduction of nasal discharge, were so obvious to the parents and the children themselves that they were happy to comply with the long-term nasal douching. When revision surgery became necessary, consent from the parents was forthcoming and given with enthusiasm. However, the aim of the present report is not to promote radical sinus surgery for children with CF. It is a long-term longitudinal study of children with CF who have undergone radical endoscopic surgery for nasal polyposis. The information may assist other clinicians in the counseling of parents before surgery.

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EUROPEAN ACADEMY OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY

The Congress of the ENT Section of the European Academy of Allergology and Clinical Immunology will be held in Ghent, Belgium, November 15-18, 2003, and will be accompanied on November 17-19, 2003, by the Fifth International Symposium on Experimental Rhinology and Immunology of the Nose. For further information, contact Congress Secretariat, Semico nv, Korte Meer 16, B-9000 Ghent, Belgium; telephone +32 9 233 86 60; fax +32 9 233 85 97; e-mail eaaci@semico.org or see the web site <http://www.semico.org/serin>.

PARAGANGLIOMA OF THE CERVICAL SYMPATHETIC TRUNK

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We report the case of a 17-year-old man who presented with left-sided Horner's syndrome. Magnetic resonance imaging revealed a spindle-shaped cervical tumor in the left paravertebral space. During operation, a tumor originating from the left sympathetic trunk was found. The histopathologic analysis showed a sympathetic paraganglioma. The sympathetic trunk is an extremely rare location for a cervical paraganglioma; only a few cases have been reported in the literature.

KEY WORDS — cervical sympathetic trunk, head and neck, Horner's syndrome, paraganglioma.

INTRODUCTION

Paragangliomas are rare, slow-growing, highly vascular tumors that arise from extra-adrenal paraganglionic tissue, which is embryologically derived from neural crest cells. The largest collection of these cells is found in the adrenal medulla, where they give rise to pheochromocytomas. The extra-adrenal paraganglionic system is grouped into 4 families — the branchiomic, the intravagal, the aortico-sympathetic, and the visceral-autonomous — based on anatomic distribution, innervation, and microscopic structure.¹ Extra-adrenal paragangliomas are found along branches of autonomic nerves throughout the body. Only 3% occur in the head and neck; 85% are found below the diaphragm, and 10% represent intrathoracic lesions.²

As summarized in a 1996 literature overview of 514 paragangliomas of the head and neck by Mickley et al,³ the most commonly found cervical paragangliomas are of branchiomic origin, with 62% occurring in the carotid body and 27% in the jugulotympanic region. Other, more unusual sites have also been reported in 2% of cases, including the nose and

paranasal sinuses, the larynx, and the orbit. Intravagal paragangliomas are found in 9% of cases.⁴⁻⁷

We report the case of a patient who presented with Horner's syndrome and whose diagnostic workup revealed a paraganglioma arising from the cervical sympathetic trunk.

CASE REPORT

In March 1999, a 17-year-old man noticed focusing problems during rifle shooting. At the same time, anisocoria had been observed by his mother. Ophthalmologic examination revealed a left-sided Horner's syndrome without any additional ocular abnor-



Fig 1. Left-sided upper and lower lid ptosis with left palpebral fissure of 6 mm versus 8 mm on right side and smaller left pupil with difference of 3 mm in dark and 1.5 mm in light were observed.



Fig 2. Focused magnetic resonance image of neck shows circumscribed, spindle-shaped mass of 3 × 1.5 × 1.3-cm size in left paravertebral space (C4-5), posterior to carotid artery, with lateral and anterior displacement of internal jugular vein (asterisk).

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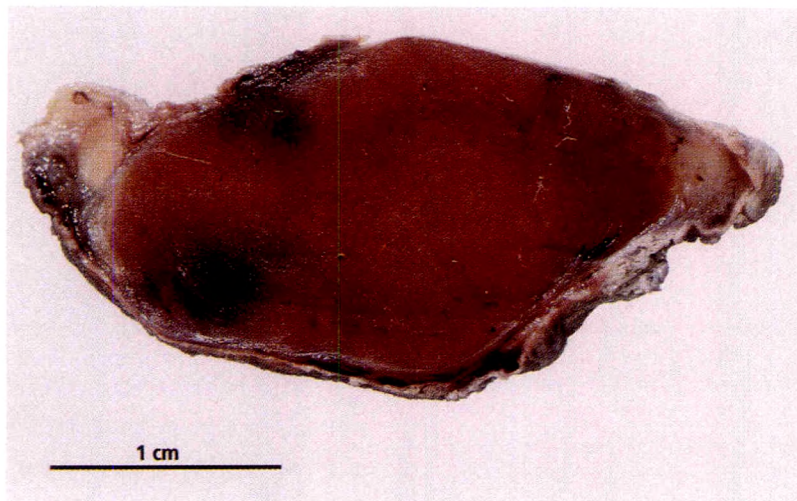


Fig 3. Gross view of soft encapsulated tumor, showing reddish brown cut surface with focal hemorrhage.

malities (Fig 1). Pharmacological testing with topical application of cocaine hydrochloride confirmed the diagnosis of a left-sided oculsympathetic paresis. Subsequently, pholedrine, an indirect sympathomimetic agent, was topically applied to both eyes and resulted in a reduction of the anisocoria, thus suggesting the site of the lesion to be preganglionic. Because the patient's preganglionic Horner's syndrome was truly isolated, with no signs of brain stem involvement, a lesion of the second neuron of the left oculsympathetic pathway was postulated, and imaging from levels C2 to T6 was ordered (Fig 2).⁸

By its clinical manifestation and the radiologic shape and location, a benign neurinoma of the sympathetic trunk or the vagal nerve was suspected. Because of the risk of postoperative vagal palsy, the patient was followed clinically and a repeat magnetic resonance imaging (MRI) scan was performed 6 months later, showing no tumor growth. Apart from

the Horner's syndrome, no other clinical symptoms were noticed.

The young patient finally wanted to have the lesion excised and subsequently underwent transcervical exploration. During operation, the tumor was found to originate from the sympathetic trunk, such that with the lesion the trunk had to be resected. The vagal nerve was not related to the tumor, and no changes of blood pressure through manipulation were noticed.

Gross findings showed a soft, encapsulated tumor with a reddish brown cut surface with areas of focal hemorrhage (Fig 3). Histopathologic analysis showed the typical morphology of a paraganglioma in direct proximity to a ganglion (Fig 4). The growth pattern of the encapsulated tumor was characterized by nests (*Zellballen*) and trabeculae of oval and polymorphonuclear cells. Immunohistochemical staining showed

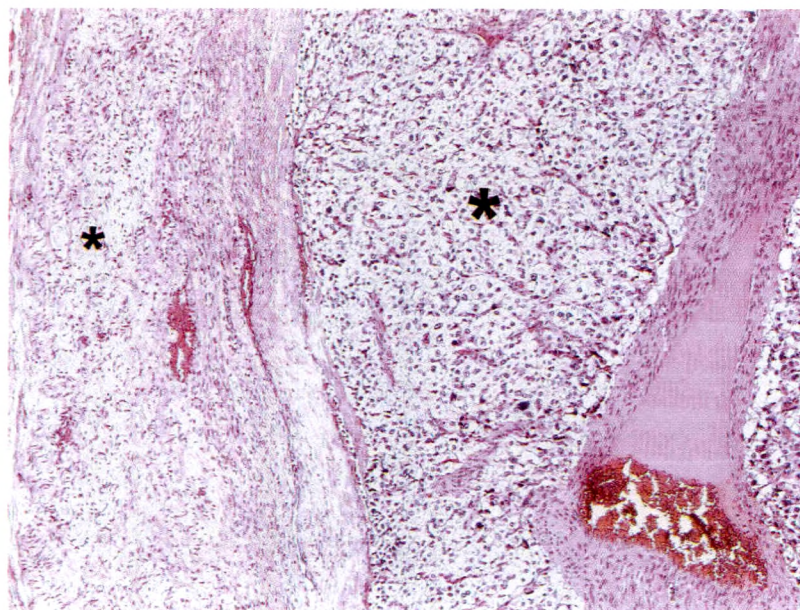


Fig 4. Hematoxylin and eosin-stained slide shows tumor growing in cords and nests (large asterisk; original $\times 40$). Tumor cells are of polymorphous appearance. Next to tumor, there is ganglion (small asterisk), separated from it by fibrous capsule.

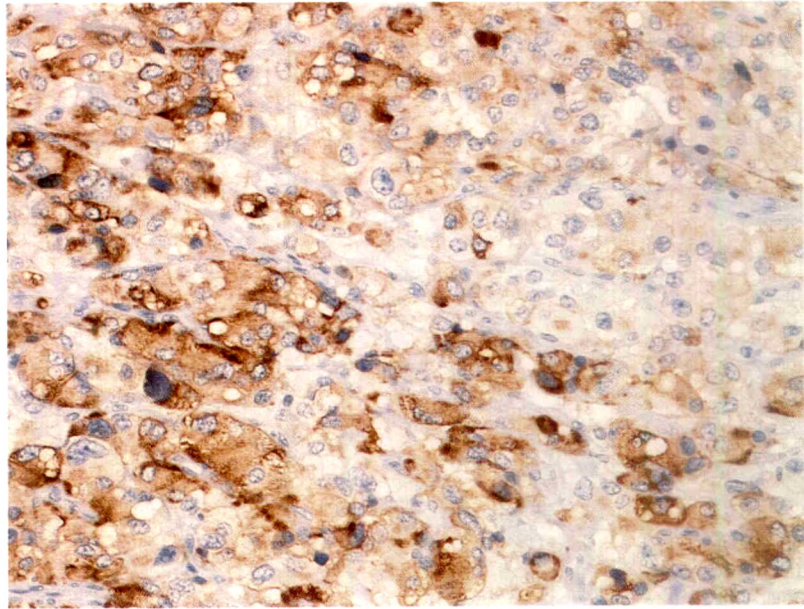


Fig 5. Immunohistochemically stained slide for chromogranin A, with strong cytoplasmic activity (original $\times 180$).

an immunoreactive pattern typical of a tumor of adrenal or extra-adrenal origin^{9,10}; positivity for synaptophysin (DAKO 1:50), chromogranin A (Enzo 1:30; Fig 5), neuron-specific enolase (DAKO 1:100), and CD 57 (HNK-1; DAKO 1:30); negativity for pancytokeratin (BMS 1:250); and positivity of sustentacular cells for S-100 (DAKO 1:500). The proliferative index (percentage of MIB [Milan Analytica 1:20]-positive cells) was less than 3%.

The postoperative course was uneventful, and the left-sided Horner's syndrome persisted.

DISCUSSION

Paragangliomas of the neck are rare tumors that may present as slowly growing masses in the lateral part of the neck. The differential diagnosis of a lesion in the lateral part of the neck includes lymphadenopathies, branchial cleft cysts, salivary gland tumors, aneurysms of the carotid artery, neurogenic tumors, and paragangliomas.

Although all paragangliomas are theoretically capable of producing catecholamines and other neuroendocrine hormones, in fewer than 2% of head and neck tumors is this clinically detectable.^{11,12} Familial paragangliomas are estimated to account for 7% to 9% of those arising in the head and neck. They are multicentric in 25% to 33%, as compared to about 2% to 10% of those that occur sporadically.¹¹⁻¹³ Malignancy of cervical paragangliomas is controversial and has been described to occur in between 2% and 12% of cases,^{4-6,11,12,14} but it is generally accepted that the malignant potential of paragangliomas cannot be predicted by histologic appearance alone.

In our case, on the basis of the combination of a

preganglionic Horner's syndrome with a spindle-shaped paravertebral tumor on MRI, the differential diagnosis included a neurinoma of either the sympathetic trunk or the vagal nerve. Surgery confirmed the origin to be in the sympathetic trunk, but the histologic analysis revealed a paraganglioma. We therefore conclude that a sympathetic paraganglioma may have the same radiologic appearance as a neurinoma.

To date, only a few cases of paragangliomas arising from the cervical sympathetic trunk have been described in the literature. Most cases of aorticosympathetic paragangliomas have been reported to occur in the posterior mediastinum or retroperitoneum.^{2,15-17}

In the literature we have found 7 reports with clear descriptions of paragangliomas arising from the cervical sympathetic trunk,^{3,11,18-22} of which 4 were catecholamine-secreting tumors.¹⁸⁻²¹ In 5 cases, an ipsilateral Horner's syndrome was described.^{3,18-20,22} Cone,¹⁸ in 1958, and Gibbs et al,²¹ in 1977, both reported a case of a child (8-year-old boy and 13-year-old girl) with a history of hypertension who had a functioning pheochromocytoma of the adrenal gland in combination with a functioning paraganglioma of the cervical sympathetic trunk. Functioning paragangliomas are often referred to as extra-adrenal pheochromocytomas. Glenner et al,¹⁹ in 1962, and Fries and Chamberlin,²⁰ in 1968, described 2 more patients (both male, 8 and 21 years old) with functioning paragangliomas of the cervical sympathetic trunk who initially presented at the hospital with a painless cervical mass and ipsilateral Horner's syndrome without a history of hypertension. The blood pressure fluctuated widely during dissection of these functioning paragangliomas; 1 patient even died of car-

diac arrest in the immediate postoperative course.¹⁹ Sobol and Dailey,¹¹ in 1990, reported 1 familial case of a nonfunctioning cervical sympathetic paraganglioma in a 28-year-old woman from a family of 4 siblings who together had 9 cervical paragangliomas. The tumor had been asymptomatic and was detected by a computed tomographic scan performed because of the positive family history. In addition, a mass on the contralateral side was found, which proved to be a carotid paraganglioma. Mickley et al,³ in 1996, and Moyer and Bradford,²² in 2001, each reported a patient (24-year-old man and 30-year-old woman) with a nonfunctioning cervical sympathetic paraganglioma, presenting with a slow-growing cervical mass and an ipsilateral Horner's syndrome without any other symptoms or positive familial history.

Furthermore, 4 cases of paragangliomas possibly related to the cervical sympathetic trunk could be found.^{12,23-25} Irons et al,¹² in 1977, mentioned 1 aorticosympathetic paraganglioma in their analysis of 116 paragangliomas of the neck without further specification. Crowell et al,²³ in 1982, presented the case of a functioning carotid paraganglioma, which they thought not to be directly related to the carotid body,

but to the autonomic ganglia in this area. Saito et al,²⁴ in 1982, described the case of a pheochromocytoma from the superior cervical sympathetic ganglion in the Japanese literature. Becker et al,²⁵ in 1992, described the case of a cervical pheochromocytoma in front of the sympathetic chain without giving anatomic details. Malignancy was not reported in these 4 cases.

Although cervical sympathetic paraganglioma is a very uncommon tumor, it should be included in the differential diagnosis of patients with preganglionic Horner's syndrome who have a mass along the sympathetic chain. The literature review revealed functioning tumors in half of the reported cases of cervical sympathetic paragangliomas. We consider the cervical sympathetic paragangliomas to belong to the sympathoadrenal neuroendocrine system, of which 25% to 86% of the tumors may be functional.¹⁰

Thus, we further conclude that measuring catecholamines in the urine is important in preventing severe intraoperative fluctuations of blood pressure whenever a paraganglioma of the sympathoadrenal system is included in the differential diagnosis.

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OTO-NEUROSURGERY AND SKULL BASE DISSECTION COURSES ON INJECTED SUBJECTS

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EFFECTS OF NIMODIPINE ON SALICYLATE OTOTOXICITY

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The purpose of this study was to examine the effects of nimodipine on salicylate ototoxicity in guinea pigs. The compound action potential (CAP) was recorded at the round window, and the cochlear blood flow (CBF) was measured simultaneously from the lateral wall of the basal turn of the cochlea by laser Doppler flowmetry. After administration of salicylate (100 mg/kg), the CAP thresholds were significantly elevated, by 5 to 20 dB ($p < .05$), and the CBF was significantly decreased ($p < .05$). After administration of nimodipine (2 mg/kg), the CAP thresholds were unchanged, but the CBF had increased significantly ($p < .05$), while systemic blood pressure had decreased significantly ($p < .05$). Simultaneous administration of both salicylate (100 mg/kg) and nimodipine (2 mg/kg) resulted in significant elevation of the CAP thresholds ($p < .05$), while the CBF did not decrease. These results suggest that nimodipine prevents the decrease in CBF induced by salicylate, but that nimodipine does not prevent the deterioration in the CAP.

KEY WORDS — cochlear blood flow, compound action potential, guinea pig, nimodipine, salicylate.

INTRODUCTION

It is well known that the administration of large doses of salicylate causes reversible mild to moderate hearing loss and tinnitus in humans. Studies have correlated serum salicylate levels to hearing loss.¹⁻⁴ A reversible bilateral hearing loss of 20 to 30 dB at all frequencies occurs as the serum salicylate level approaches 300 mg/L.¹ Salicylate administration to guinea pigs causes relatively minor histologic changes to the outer hair cells of the cochlea,⁵ such as swelling.⁶ Dieler et al⁶ speculated that this swelling of the outer hair cells could be mediated by changes in the intracellular calcium concentration. Salicylate produces threshold elevations of 20 to 30 dB, and abnormal neural synchronization has been observed in cat primary auditory cortex upon administration of sodium salicylate.⁷ We have previously suggested that changes in calcium ion (Ca^{2+}) channel conductance and the free intracellular Ca^{2+} concentration result in abnormal neural synchrony in cortical cells.⁷ Abnormal neural synchrony was postulated as a potential correlate of tinnitus.^{8,9}

Nimodipine, an L-type Ca^{2+} channel antagonist, is lipophilic and readily crosses the blood-brain barrier.¹⁰ Initially, nimodipine was used to prevent cerebral artery spasms following subarachnoid hemorrhage¹⁰ and to reduce neurologic deficits following ischemic stroke.¹¹ In addition to its antivasoconstrictor effect on the cerebral vessels, nimodipine has been used to treat tinnitus¹² and Meniere's disease.¹³ In

behavioral studies in the rat, the tinnitus induced by salicylate or quinine was totally abolished by nimodipine.^{14,15} Conversely, there have been reports that nimodipine is ineffective in the treatment of noise-induced hearing loss¹⁶ and the threshold elevation of the auditory brain stem response (ABR) induced by salicylate.¹⁷ Moreover, Bobbin et al¹⁸ reported ototoxic effects of nimodipine on the compound action potential (CAP), cochlear microphonics, and the summing potential.

Although the effects of salicylate on the CAP threshold are now well understood, the effects of nimodipine on salicylate ototoxicity remain controversial. In the study reported here, we used the electrocochleogram to examine the effect of nimodipine on salicylate ototoxicity. In addition, we simultaneously measured the cochlear blood flow (CBF). The CBF is related to cochlear function, because it controls the transport of oxygen and nutrients to (and removal of metabolites from) the inner ear.

MATERIALS AND METHODS

Animal Preparation. Adult male Hartley guinea pigs weighing 300 to 420 g received an intraperitoneal injection of sodium pentobarbital at a dose of 25 mg/kg body weight. After 15 minutes, Xylocaine (a mixture of lidocaine hydrochloride and epinephrine) was injected subcutaneously around the trachea, and a tracheostomy was performed. The animals were paralyzed by an intramuscular injection of Relaxin (suxamethonium chloride) at a dose of 50 mg/kg

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body weight and ventilated artificially. After head shaving and tracheostomy, Xylocaine was injected subcutaneously at the right retroauricular region, and then the right retroauricular skin was incised to expose the round window. The left carotid artery was cannulated and connected to a pressure transducer for continuous blood pressure (BP) measurement with MacLab software (AD Instruments Pty Ltd, Sydney, Australia), while the left jugular vein was cannulated to compensate for the serum volume loss. We used heart rate and BP measurements to monitor the level of anesthesia. The guinea pig temperature was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a thermostatically controlled unit (BWT-100, Bio Research Ltd, Nagoya, Japan). Animals were divided into 3 groups: salicylate (group S; $n = 7$), nimodipine (group N; $n = 8$), and salicylate and nimodipine (group SN; $n = 7$). The effects of the drugs in each group were assessed by comparison of measurements made before and after drug administration. At the end of the experiment, the animals were painlessly sacrificed by an overdose of sodium pentobarbital. The care and use of the animals were approved by the Animal Care and Use Committee of St Marianna University.

Acoustic Stimulus Presentation. Sound stimuli were presented by a loudspeaker (JBL-2450H, JBL Inc, Northridge, California) placed 40 cm from the tested ear. Calibration and monitoring of the sound pressure waveforms was performed with a condenser microphone (NA-41, Rion Ltd, Tokyo, Japan) positioned above the animal's head. The CAP audiograms were measured with tone bursts (1-ms rise-fall time, 5-ms plateau) at 2, 4, 8, and 16 kHz. Rectangular electric stimuli of 0.1-ms duration were delivered to the transducer to produce the acoustic click. Both stimuli were generated by a masking generator (DPS-727, Dia Medical System Co, Ltd, Tokyo) and a stimulator (DPS-1100D, Dia Medical System Co, Ltd).

Recording Strategy. The recording sequence was composed of 2 parts: CAP and CBF. The CAP recording and the CBF measurement were performed in the right ear in all animals. Both an electrode for CAP recording and a probe for CBF measurement were kept in the same place before and (over 90 minutes) after administration of drugs. After confirming the stability of the CAP, CBF, and BP, we recorded the preadministration data of these parameters to serve as controls. It usually took approximately 60 minutes to obtain a stable recording, after which each drug was slowly administered. Sodium salicylate at a dose of 100 mg/kg was administered intravenously in group S. This dosage is the same as that used in our previous study on the cortex.⁷ The dosage of nimodipine was determined by a preliminary study that assessed its effects on the ABR. The ABR threshold to

click stimuli in 2 guinea pigs was elevated by more than 10 dB when 4 mg/kg of nimodipine was administered, while 2-mg/kg and 1-mg/kg doses did not cause any threshold elevation. Therefore, nimodipine at a dose of 2 mg/kg was administered subcutaneously in group N. Sodium salicylate at a dose of 100 mg/kg was administered intravenously and nimodipine at a dose of 2 mg/kg was administered subcutaneously in group SN. The CBF and BP were recorded for 90 minutes after the administration of each drug. The CAP recording was performed both before (as a control) and at approximately 30 minutes after the administration of each drug.

CAP Recording. A silver ball electrode was placed on the round window membrane. Electrical responses were amplified 10,000 times by a biophysical amplifier (AVB-11, Nihon Kohden Ltd, Tokyo), bandpass-filtered from 0.08 Hz to 10 kHz, and then fed to a Macintosh computer for averaging on the MacLab system. The activity was sampled at 20 kHz for 10 ms after stimulus onset. The recording sequence was composed of 2 parts, using stimuli of tone bursts and clicks.

CBF Recording. The mucosal vessels on the surface of the exposed cochlea were gently removed with cotton pledgets. The 0.5-mm-diameter "needle" probe of a laser Doppler flowmeter (BRL-100, Bio Research Ltd) was held securely in a micromanipulator while the probe tip was positioned against the lateral wall of the basal turn of the cochlea to minimize artifactual laser Doppler flowmetry signals caused by movement between the probe tip and the bone surface of the cochlea. A light coating of petroleum jelly was used between the probe tip and the bone surface of the cochlea in order to ensure constant optical coupling of the laser light and to prevent the accumulation of fluid and extraneous blood beneath the probe tip. The laser Doppler flowmeter averaging time was set to 1 second. The BP and CBF signals were digitally (and simultaneously) recorded with the MacLab system.

Data Analysis. The CAP thresholds were determined for each tone burst stimulus. The CAP amplitude is measured from the onset to the peak of the CAP. The CAP was recorded at approximately 30 minutes after the administration of each drug. We were able to record the CAP before and after administration of drugs in all 22 guinea pigs without changing the electrode position. We were not able to record the CBF in 1 guinea pig in group N and 1 guinea pig in group SN, because of loss of the CBF signal. Consequently, CBF data were obtained from 7 guinea pigs in groups S and N and from 6 guinea pigs in group SN. The ratio of the CBF and BP data (CBF/BP ratio) was calculated. The values at the 0-minute point rep-

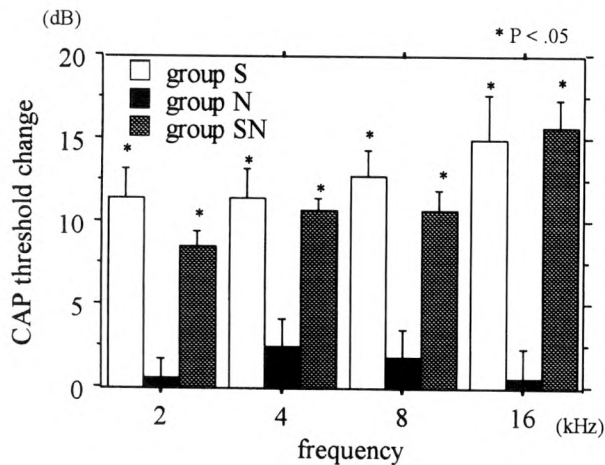


Fig 1. Relationships between change in compound action potential (CAP) threshold and tone burst frequency. Means $\pm$ 1 SD (vertical bar) are indicated. Group S — salicylate group ($n = 7$); group N — nimodipine group ($n = 8$); group SN — salicylate and nimodipine group ($n = 7$). Asterisks indicate significant change ($p < .05$) following drug administration.

represented the preadministration data. Each parameter is expressed as the mean of the values measured for 5 minutes around each indicated time.

All tests were performed with the StatView 5.0J package for MS-Windows (SAS Institute Inc, Cary, North Carolina). Statistical tests for the data obtained before and after administration of each drug were based on the Wilcoxon signed rank test and Mann-Whitney U-test. Changes at the $p < .05$ level were considered significant.

RESULTS

Changes in CAP Thresholds. Figure 1 illustrates the changes in CAP threshold induced by each drug. The thresholds of group S were elevated by 5 to 20 dB after salicylate administration. The means and standard deviations of the threshold elevation to each tone burst stimulus were as follows ($n = 7$): 2 and 4 kHz, 11.4 ± 4.8 dB; 8 kHz, 12.9 ± 3.9 dB; and 16 kHz, 15.0 ± 7.1 dB. These changes were all statistically significant (Wilcoxon signed rank). The threshold elevations increased with increasing tone burst frequency; however, these frequency-related differences did not reach the level of statistical significance (Wilcoxon signed rank test). The CAP thresholds of group N were not changed significantly after nimodipine administration (Wilcoxon signed rank test). The means and standard deviations of the threshold elevation to each tone burst stimulus were as follows ($n = 8$): 2 kHz, 0.63 ± 3.20 dB; 4 kHz, 2.50 ± 4.63 dB; 8 kHz, 1.88 ± 4.58 dB; and 16 kHz, 0.63 ± 4.96 dB. The CAP thresholds of group SN were elevated by 5 to 25 dB after simultaneous application of both salicylate and nimodipine. The means and standard de-

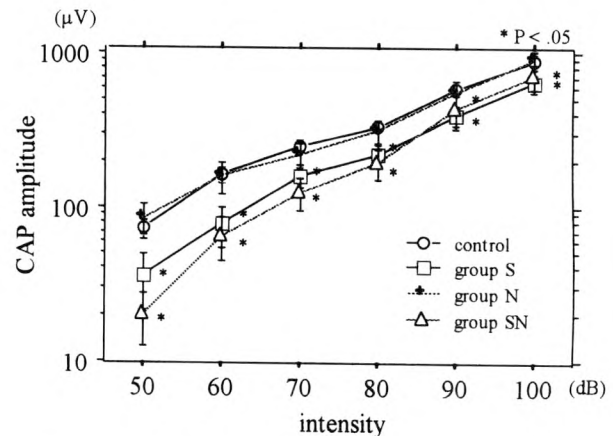
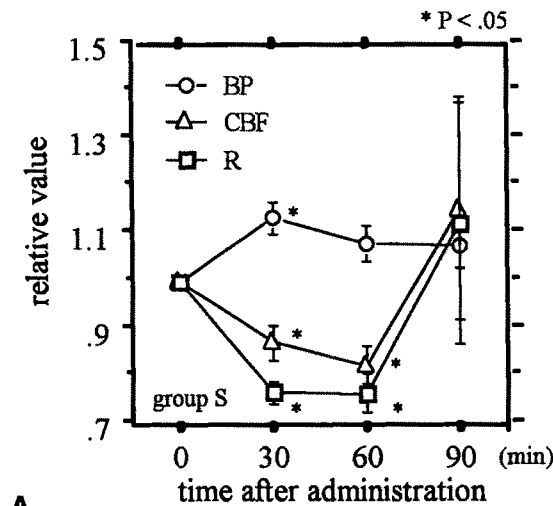


Fig 2. Relationships between stimulus intensity and CAP amplitude. Means $\pm$ 1 SE (vertical bar) are indicated. Six different stimulus intensities were used for this analysis. For control group, $n = 22$; group S, $n = 7$; group N, $n = 8$; and group SN, $n = 7$. Asterisks indicate significant change ($p < .05$) following drug administration.

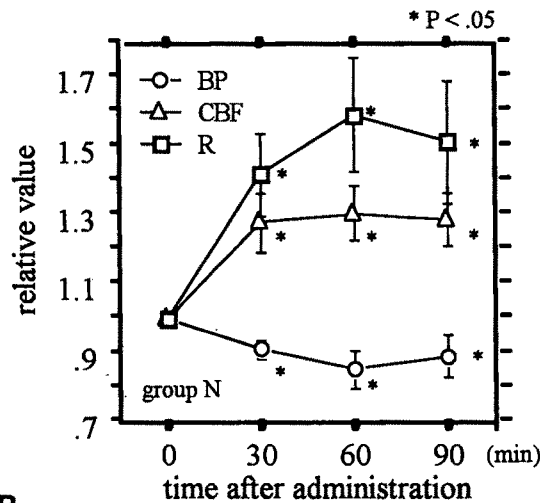
viations of the threshold elevation to each tone burst stimulus were as follows ($n = 8$): 2 kHz, 8.57 ± 2.44 dB; 4 kHz, 10.71 ± 1.89 dB; 8 kHz, 10.71 ± 3.45 dB; and 16 kHz, 15.7 ± 4.50 dB. These changes in group SN were all statistically significant (Wilcoxon signed rank test). The difference between the threshold elevations in groups S and SN was not statistically significant at any of the frequencies measured (Mann-Whitney U test). In contrast, the difference between the threshold elevations in groups N and SN was statistically significant at all frequencies (Mann-Whitney U test).

Relationship Between Stimulus Intensity and CAP Waveform. Figure 2 depicts the relationship between the stimulus intensity and the CAP amplitude before and after drug administration. The CAP amplitude decreased after salicylate administration in all animals. The differences in the amplitudes measured before and after salicylate administration at 6 intensities were all statistically significant (Wilcoxon signed rank test). The mean CAP amplitudes decreased after administration of nimodipine, but the differences at the 6 intensities did not reach the level of statistical significance (Wilcoxon signed rank test). In contrast, group SN exhibited a statistically significant amplitude decrease at all 6 intensities (Wilcoxon signed rank test).

Changes in CBF. The CBF had decreased by approximately 13%, while the BP had increased by approximately 14% at 30 minutes after administration of salicylate. These trends continued until 60 minutes after administration, as shown in Fig 3A. Both the BP and the CBF had returned to the control level 90 minutes after drug administration. Consequently, the CBF/BP ratio had decreased by about 24% at 30 and 60 minutes, before returning to close to the control

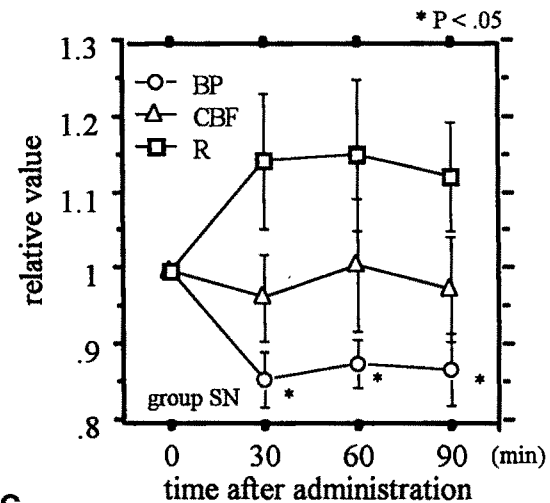


A



B

Fig 3. Changes in blood pressure (BP), cochlear blood flow (CBF), and CBF/BP ratio (R) are plotted as function of time after administration of each drug as mean $\pm$ 1 SE (vertical bar). Changes in **A**) group S ($n = 7$); **B**) group N ($n = 7$); and **C**) group SN ($n = 6$) are shown. Each parameter is expressed relative to its value prior to administration of drug. Asterisks indicate significant difference as compared with level prior to administration of drugs ($p < .05$).



C

level. Statistical analysis revealed a significant change in all measured parameters at 30 and 60 minutes (except for BP at 60 minutes) as compared with preadministration levels, while no significant differences were observed at 90 minutes (Wilcoxon signed rank test).

The CBF had increased by approximately 30%, while the BP had decreased by approximately 10% to 15% at 30, 60, and 90 minutes after administration of nimodipine, as shown in Fig 3B. Consequently, the CBF/BP ratio maintained a value that was approximately 40% to 60% larger than the preadministration value. Statistical analysis revealed significant changes in all measured parameters at all indicated times as compared with preadministration levels (Wilcoxon signed rank test).

There were no marked changes in the CBF after simultaneous administration of both salicylate and nimodipine, as shown in Fig 3C. The BP decreased by approximately 15% and the CBF/BP ratio increased by approximately 15% as compared to the preadministration level. Statistical tests revealed a

significant change in BP only at 30, 60, and 90 minutes after simultaneous administration of both salicylate and nimodipine (Wilcoxon signed rank test).

DISCUSSION

Threshold elevations of CAP of 5 to 20 dB were observed in all animals after salicylate application. This is consistent with our previous study on the cortex.⁷ The CAP waveform was reduced in amplitude at both high and low stimulus intensities. The CBF was decreased after administration of salicylate. The fact that the changes in CAP and CBF were similar after administration of salicylate suggests that the mechanisms underlying the changes in both CAP and CBF are the same. The regulation of the diameter of the spiral modiolary artery may play a major role in the regulation of CBF and hence in tissue oxygenation, since the spiral modiolary artery provides the main blood supply to the cochlea. The spiral modiolary artery contains voltage-dependent, nifedipine-sensitive Ca^{2+} channels that are involved in the maintenance of basal vascular tone, as well as in the mediation of potassium ion- and norepinephrine-induced

contraction.¹⁹ The changes in Ca^{2+} channel conductances may alter the basal vascular tone and lead to changes in CBF. Considering these and other potential mechanisms underlying electrophysiological changes and decreases in CBF,^{6,7,10,11,19} we believe that the Ca^{2+} channels may well be responsible for the effects observed in the present study.

The CAP thresholds did not deteriorate after administration of nimodipine. The CAP input-output function was almost the same after the administration of nimodipine as before. This finding is consistent with our preliminary study on the ABR. A significant increase in CBF was observed after administration of nimodipine. The animals in group SN exhibited no significant changes in CBF after administration of the drugs, but significant decreases in CBF were observed in group S. This finding suggests that nimodipine prevents the decrease of CBF induced by salicylate. Coleman et al²⁰ reported that nimodipine is a very promising agent for inducing increases in CBF — a finding that supports its potential usefulness in the treatment of otologic disease. The effect of nimodipine on salicylate ototoxicity was limited to changes in CBF, since administration of nimodipine did not prevent the CAP deterioration induced by salicylate. There are 2 potential reasons for this differ-

ence. One relates to the dosage of nimodipine. We observed ABR threshold elevation in our preliminary experiment when we administered 4 mg/kg of nimodipine, so we consider that 2 mg/kg is the highest dose that does not induce nimodipine ototoxicity. In addition, this dosage of nimodipine is the same as that used in other reports.^{14,15,17} The above discrepancy between the effects of nimodipine is therefore unlikely to be the result of the dosage. The other possibility is that the effect of salicylate on CAP deterioration might not be induced through a decrease in CBF. Moreover, changes in Ca^{2+} channels are unlikely to be the cause of the deterioration in hearing induced by salicylate. In previous reports, the protective effect of nimodipine against salicylate ototoxicity was limited to tinnitus.^{10,11} Kay and Davies¹⁷ reported that nimodipine was ineffective against the deterioration of hearing caused by salicylate. These reports are consistent with our present results. It is possible that the effect of salicylate on CBF and tinnitus arises through changes in Ca^{2+} channels and is prevented by the administration of a Ca^{2+} channel antagonist, and that the effect on hearing is not provoked through changes in Ca^{2+} channel conductance. We speculate that the mechanisms underlying tinnitus and hearing impairment from salicylate administration are not exactly the same.

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GLUCOCORTICOID-INDUCED DIFFERENTIAL EXPRESSION OF THE SIALYLATED AND NONSIALYLATED LEWIS^a EPITOPES AND RESPECTIVE BINDING SITES IN HUMAN NASAL POLYPS MAINTAINED UNDER EX VIVO TISSUE CULTURE CONDITIONS

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We characterized the anti-inflammatory effects of budesonide on the expression of adhesion molecules involving Lewis^a (Le^a) epitope, its sialylated derivative (sLe^a), and their respective binding sites in human nasal polyposis. By computer-assisted microscopy, we quantitatively characterized the level of histochemical expression of L- and P-selectins, sialylated and nonsialylated Le^a epitopes, and their respective binding sites in both surface epithelium and glandular epithelium of human nasal polyps obtained from surgical resection, maintained under ex vivo tissue culture conditions for 24 hours, and treated or not with budesonide. Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) were chosen as methodological controls, because data already published in the literature clearly indicated budesonide-mediated effects on ICAM-1 and VCAM-1 levels of expression. The present data show that budesonide significantly modified the levels of expression of ICAM-1 and VCAM-1, and to a lesser extent that of P-selectin, in the surface and glandular epithelia. Budesonide markedly decreased the levels of expression of the binding sites for both Le^a and sLe^a, while those of Le^a and sLe^a remained globally unchanged. In conclusion, the present study documents that glucocorticoid-induced effects can encompass receptors for Le^a epitopes different from E- and P-selectins on epithelial cells of human nasal polyps.

KEY WORDS — budesonide, glucocorticoid, inflammation, Lewis antigen, nasal polyp, neoglycoconjugate, selectin.

INTRODUCTION

Nasal polyps constitute a common manifestation of diseases affecting the respiratory mucosa.¹ Such a disorder is known to occur in association with other forms of rhinitis, aspirin idiosyncrasy, cystic fibrosis, and Kartagener's syndrome.² Histologically, these polyps are characterized by chronic inflammation in which eosinophilic infiltration predominates. Corticosteroids applied as systemic and topical treatments have demonstrated symptomatic efficacy.³ They reduce rhinitis symptoms, improve nasal breathing, and reduce both the size and recurrence of nasal polyps.⁴ Glucocorticoids exert their anti-inflammatory effects by increasing the transcription of anti-inflammatory genes, including, for example, lipocortin-1 and the leukocyte inhibitory protein, and by decreasing the transcription of inflammatory genes, including, for example, granulocyte-macrophage colony-stimulating factor, eotaxin, several cytokines,

including interleukin (IL)-2, IL-3, IL-4, IL-5, IL-11, IL-13, and tumor necrosis factor α , which play notable roles in eosinophil pathobiology.⁵⁻⁷

In addition to cytokines and chemokines, adhesion molecules are required to orchestrate the migration and homing of inflammatory cells in inflammatory sites. The selectin family of adhesion molecules mediates the initial attachment of leukocytes to venular endothelial cells (a process referred to as rolling) before their firm adhesion (mediated by integrins and their ligands, ie, intercellular adhesion molecules [ICAMs] and vascular cell adhesion molecules [VCAMs]) and diapedesis (mediated by platelet-endothelial cell adhesion molecule 1) at tissue injury and inflammation sites.^{8,9} Evidently, glucocorticoids can also modulate the expression of various types of adhesion molecules. Data published in the literature mainly emphasize the glucocorticoid-mediated down-regulatory effects at cell adhesion molecule lev-

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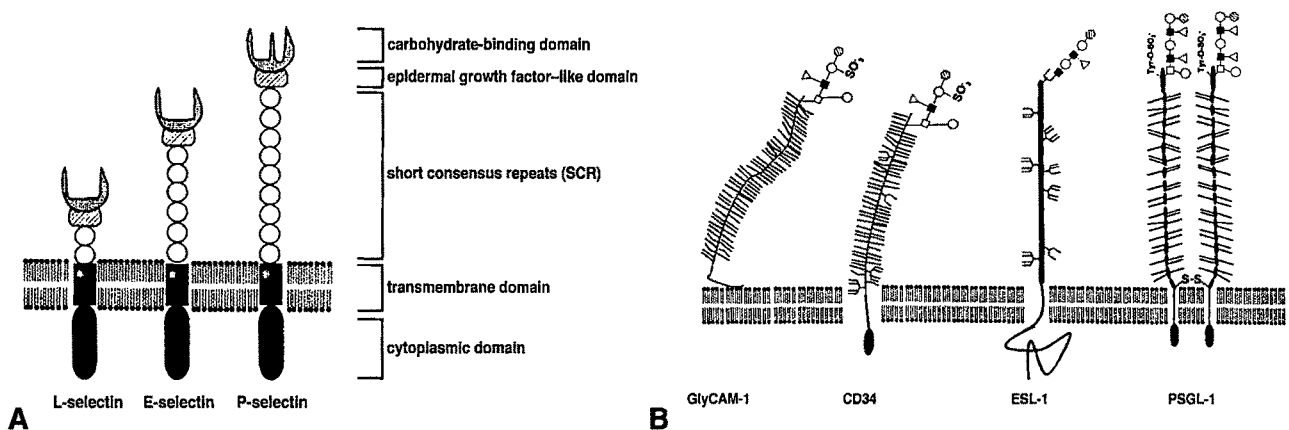


Fig 1. Structural characteristics of A) selectins and B) certain selectin ligands. Modular arrangement of 3 members of selectin family starts with extracellular N-terminal C-type lectin domain and epidermal growth factor-like sequence (A). Two L-selectin, 6 E-selectin, and 9 P-selectin consensus repeats homologous to complement-binding domain, transmembrane region, and cytoplasmic C-terminal domain complete modular arrangement of this subgroup of C-type lectins (A). B gives schematic illustration of natural selectin ligands carrying carbohydrate moieties outlined in Fig 2. These carbohydrate moieties are crucial for binding to selectin carbohydrate recognition domains (A), and are presented by various glycoproteins serving as selectin ligands (B). GlyCAM-1 or CD34, two heavily O-glycosylated mucin-like proteins, are *in vitro* ligands of L-selectin. Both are expressed constitutively on high endothelial venules of peripheral lymph nodes. Whereas GlyCAM-1 lacks transmembrane domain and is most probably secretory protein, CD34 is type 1 transmembrane glycoprotein of 140 amino acids. Glycans of ESL-1 serve as ligand for E-selectin. In contrast to mucin-type ligands with abundant glycosylation, this glycoprotein is hardly decorated by N-glycans. P-selectin glycoprotein ligand-1 (PSGL-1) is disulfide-bridge-linked dimeric type 1 transmembrane protein of 402 amino acids isolated from human leukocytes. Symbols used to describe carbohydrate moieties (ie, Lewis antigens; see Fig 2) located at tips of selectin ligands are open dots for D-galactose, open squares for N-acetyl-D-galactosamine (GalNAc), hatched dots for N-acetyl-D-neuraminic acid (Sia), black squares for N-acetyl-D-glucosamine (GlcNAc), and open triangles for L-fucose (Fuc). Single spindles on backbone of protein carrier represent O-linked glycans, and double- or triple-branched chains represent N-linked glycan antennae. Sizes of individual parts of scheme cannot be true to scale if structural details of crucial sites on glycan part, their density, and their arrangement on carrier and backbone itself are to be illustrated in one Figure. (Adapted from Kaltner and Stierstorfer.⁹)

els.¹⁰⁻¹² With relevance for the selectin system, E- and L-selectin down-regulation has been reported as a consequence of glucocorticoid treatment,¹³⁻¹⁶ and — in a broader context — inflammation-dependent induction of glycoforms of α 1-acid glycoprotein with sialylated Le^x-determinants also points to regulation at the level of the carbohydrate part of this recognition system.¹⁷ These data underscore the importance of analyzing this recognition system with respect to the inflammatory response in nasal polyps.

There are 3 known selectins, ie, L-selectin (leukocyte adhesion molecule 1 [LECAM-1]), E-selectin (endothelial-leukocyte adhesion molecule 1 [ELAM-1]), and P-selectin (GMP-140).^{8,9} These selectins share a modular display (Fig 1A⁹) that includes a C-type lectin domain.¹⁸ Their carbohydrate recognition domains (Fig 1A) bind specifically to fucosylated and sialylated/sulfated oligosaccharides presented on various glycoproteins (Fig 1B) and belonging to the Lewis family of antigens (Fig 2¹⁹). The sialyl Lewis^{a/x} (sLe^{a/x}) oligosaccharides (Fig 2) are supposed to serve a ligand structure common to all 3 selectins, with sulfate substitutions and changes in the topology of presentation markedly affecting the target avidity.¹⁶⁻²⁰ As indicators to pinpoint regulation in this system, selectin-specific antibodies have already

proven their value. Moreover, with antibodies specific for Le determinants, a modulation in glycan production can readily be monitored. In addition, we introduce an innovative class of synthetic probes for assessing glycan modifications in this respect.²¹

The aim of the present study was to investigate whether a glucocorticoid (here, budesonide) is able to significantly modify the levels of expression of selectins and selectin ligands in human nasal polyps, a model of eosinophil-mediated inflammation. For monitoring, organotypical cultures of human nasal polyps obtained from surgical resection were subject to study. Using computer-assisted microscopy,^{22,23} we quantitatively characterized immunohistochemically the levels of P- and E-selectin expression in human nasal polyp organotypical cultures either treated with budesonide or left untreated. We chose ICAM-1 and VCAM-1 as internal controls of the method. As outlined above, in addition to the 2 endothelial selectins, the levels of expression of Le^a (Lewis^a) and sLe^a (sialyl-Lewis^a) epitopes were immunohistochemically analyzed with specific and commercially available antibodies. The capacity of cells to bind distinct sugar epitopes was — in addition to immunohistochemical analysis of 2 selectins — determined by carrier-immobilized carbohydrate

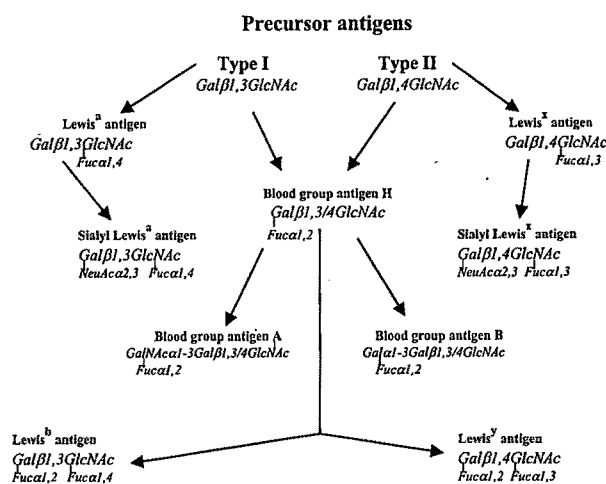


Fig 2. Schematic illustration of biochemical pathways leading to sialylated (or sulfated) Lewis antigen motifs that constitute active part of ligands in case of selectins (see Fig 1). Each arrow indicates biochemical route for structural extension of respective determinant. Symbols used are Gal for D-galactose, Glc for D-glucose, NAc for N-acetyl, NeuAc for N-acetyl-D-neuraminic (sialic) acid, and Fuc for L-fucose. Lewis^a and Lewis^x antigens can be synthesized when α 1,3-fucosyltransferase is present. In contrast, erythroid cells, which lack α 1,3-fucosyltransferase, express α 1,2-fucosyltransferase. Resultant H-type oligosaccharides (O blood type) are converted into A and B blood group antigens by addition of α 1,3-linked N-acetylgalactosamine or galactose through erythroid cell-specific expression of 2 unique glycosyltransferases.¹⁹

structures as the ligand part on a histochemically inert backbone.²¹ This type of tool extends the scope of immunohistochemistry and might provide further insights into regulation within this system of molecular recognition with relevance for inflammatory reactions and their therapeutic control.

MATERIALS AND METHODS

Clinical Data and Histopathologic Characteristics. Medical records and samples of nasal polyps were obtained from 10 patients, with the polyposis diagnoses established according to the classification that we reported earlier.²⁴ The 10 nasal polyps in the present study involved massive polyposis and came from 5 allergic and 5 nonallergic patients. The allergic versus nonallergic status of the patients was determined on the basis of the skin prick test and the total and specific IgE expression.²⁵

Ex Vivo Tissue Cultures of Human Nasal Polyps. The 10 nasal polyps were maintained under organotypical culture conditions in a manner identical to the method that we have described for human brain,²⁶ prostate,²⁷ and colon²⁸ tissue. Briefly, after surgical removal, the nasal polyp specimens were rinsed twice in minimal essential medium (MEM; Gibco-Life Technologies, Merelbeke, Belgium) and immediately cut in half. One half was used for histopathologic

diagnosis, and the other was immediately divided into pieces measuring approximately 2 to 3 mm³. In order to overcome the problem of biological heterogeneity, each experimental condition included 10 to 15 randomly selected tissue pieces for each nasal polyp. These pieces were cultured for 24 hours in 30 mm (diameter) $\times$ 10 mm (depth) Petri dishes (Nunc-Life Technologies, Merelbeke) containing 3 mL of culture medium, ie, MEM supplemented with a mixture of 0.6 mg/mL glutamine (Gibco), 200 IU/mL penicillin (Gibco), 200 IU/mL streptomycin (Gibco), and 0.1 mg/mL gentamicin (Gibco). The effects of 3 concentrations (10, 50, and 250 ng/mL) of budesonide (Rhinocort, AstraZeneca, Brussels, Belgium) were studied. For this purpose, budesonide was dissolved in dimethyl sulfoxide, with a final dimethyl sulfoxide concentration of less than 0.1%. An equal volume of this solvent was added to the control cultures to control for solvent effects. After 24 hours of culture, the samples were fixed in buffered formalin for 5 days. After paraffin embedding, 5- μ m sections were prepared for quantitative histochemical analysis as detailed below.

Immunocytochemistry and Glycohistochemistry. The histochemical procedures were carried out as detailed previously.^{22,23} Briefly, for each treatment condition, 10 to 15 tissue pieces were embedded in the same paraffin block, from which 5- μ m-thick sections were cut. These sections were subjected to processing with the various antibodies detailed below and with the biotinylated neoglycoconjugates carrying Lewis antigens. Incubation with the probe-containing solutions was carried out at 25°C $\pm$ 1°C for 30 minutes at a concentration of 10 μ g/mL. The extent of the specifically bound markers was visualized by avidin-biotin-peroxidase complex (ABC) kit reagents (Vector Labs, Burlingame, California), with diaminobenzidine-hydrogen peroxide as the chromogenic substrate.

The control reactions for each of the 6 antibodies under study included the omission of the incubation step in the case of the primary antibody, and also the preincubation of the antibody with the antigen. The sugar determinants and the specificity of each antibody were checked by Western blotting (data not shown). The neoglycoconjugates were synthesized and biotinylated as described in detail elsewhere.^{29,30} The control reactions for the application of the biotinylated probes included competitive inhibitions to ascertain sugar specificity, and the omission of the incubation step with a labeled marker served to exclude any staining by the binding of kit reagents such as the mannose-rich glycoproteins horseradish peroxidase and avidin. Counterstaining was carried out

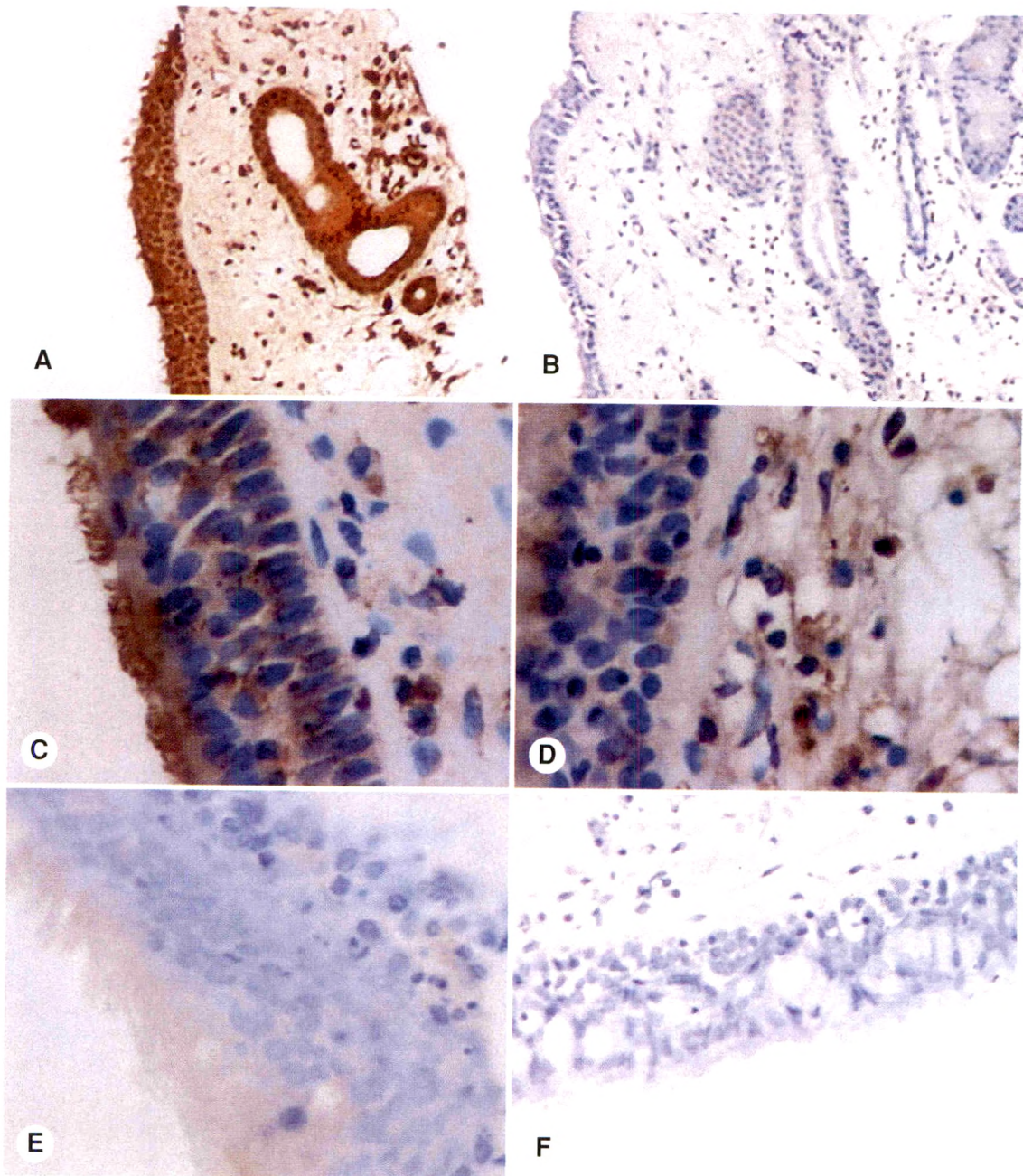


Fig 3. Morphological illustrations (original $\times 100$) of immunohistochemically detected expression of **A)** E-selectin and **B)** P-selectin in 2 pieces of nasal polyp (from allergic patients) maintained for 24 hours under organotypical culture conditions in absence of budesonide (control). **C,D)** Immunohistochemical evidence of presence of intercellular adhesion molecule 1 (ICAM-1) in surface epithelium (original $\times 400$) of 2 pieces of nasal polyp (from allergic patients) maintained for 24 hours under **C)** control (without budesonide treatment) and **D)** 250 ng/mL budesonide-treated organotypical culture conditions. **E,F)** Results obtained under same experimental conditions as in **C)** (without budesonide) and **D)** (with budesonide), but presenting glycohistochemical evidence of presence of binding sites for Lewis antigen a (Le^a) (original $\times 400$) in 2 pieces of nasal polyp from nonallergic patients.

with hematoxylin (Fig 3).

The origins and dilutions of each of the 6 commercial antibodies were as follows.

1. *Anti-Le^a*. The anti-Le^a monoclonal antibody was obtained from a BALB/c mouse that had been immunized with mucin isolated from ovarian cyst

fluid, and the splenocytes were fused with mouse myeloma SP2/0 cells (clone 7LE; Seikagaku Corp), dilution 1/100.

2. *Anti-sLe^a*. The anti-sLe^a monoclonal antibody was obtained from spleen cells of a BALB/c mouse immunized with human colon cancer cells (the CoLo-201 cell line). This antibody preferentially recognizes

the 2→3 sLe^a antigen (Fig 2), but also reacts with other structurally related sialylated type I chain carbohydrate antigens (Fig 2; code 2D3; Seikagaku Corp), dilution 1/100.

3. *Anti-E-selectin (CD62E)*. The anti-human E-selectin polyclonal antibody was produced in goats immunized with purified, CHO (Chinese hamster ovary)-cell-derived, recombinant human E-selectin. The BBA18 clone used here reacts with E-selectin only (code BBA18; R&D Systems Europe Ltd, Abingdon, England), dilution 1/500.

4. *Anti-P-selectin (CD62P)*. The anti-human P-selectin monoclonal antibody is derived from the AK4 hybridoma. The immunogen was a human platelet glycoprotein membrane fraction (100 to 200 kd) immunodepleted of glycoprotein IIb-IIa (CD41). This antibody recognizes the human P-selectin antigen expressed by activated platelets and endothelial cells (code S5924; Sigma, St Louis, Missouri), dilution 1/100.

5. *Anti-VCAM-1 (CD106)*. The anti-human VCAM-1 polyclonal antibody was produced in a goat immunized with purified, CHO-cell-derived, recombinant VCAM-1 (code BBA19; R&D Systems Europe Ltd), dilution 1/200.

6. *Anti-ICAM-1 (CD54)*. The anti-human ICAM-1 monoclonal antibody was produced against ICAM-1 immunogen originating from the Raji cell line (code 1132; Sanbio, Uden, the Netherlands), dilution 1/200.

Computer-Assisted Microscopy for Quantitative Histochemistry. In order to characterize the extent of staining dependent on each of the 8 histochemical markers, 2 quantitative variables were determined by means of a Samba 2005 computer-assisted microscope system (Samba Technologies, Grenoble, France) with a 40× (aperture 0.50) magnification lens. The labeling index (LI) refers to the percentage of tissue area specifically stained by a given histochemical marker. The mean optical density (MOD) denotes staining intensity computed on histochemically positive areas only. Both the standardization procedures dealing with the manner in which we used the computer-assisted microscopy and the way in which we used the computer-assisted system to quantify the histochemical staining have been detailed elsewhere.^{22,23} A negative histologic control slide (standardized processing without the incubation step involving the primary antibody or the biotinylated neoglycoconjugate) was analyzed for each specimen under study. The software used on the computer-assisted microscope automatically subtracted the LI and MOD values of the negative control sample from each corresponding positive one.

As detailed previously,²⁴ the measurements were directed separately to 3 distinct histologic structures of human nasal polyps, ie, the surface epithelium, the glandular epithelium, and the connective tissue. In the present study, we analyzed 2 of these 3 structures only, ie, the 2 different epithelia. The first reason for omission of quantitative analysis in the connective tissues was to eliminate any bias in the quantitative data. Indeed, although a relatively constant thickness could be obtained in the epithelia (ie, $5 \pm 1 \mu\text{m}$), maintenance of this parameter could not reliably and consistently be ensured in connective tissue (data not shown). The second reason was the focus of interest on epithelial cell-leukocyte rather than connective tissue-leukocyte interactions, as is detailed in the Discussion. Ten fields of 60,000 to 120,000 μm^2 each were scanned at each experimental condition for each type of epithelium and for each nasal polyp.

Statistical Analyses. Statistical comparisons of the data were carried out with Student's *t*-test (1-way variance analysis for 2 groups) after a check of the equality of variance by means of the Levene test, and the normal distribution fitting of the data by means of the χ^2 test of goodness of fit. When these parametric conditions were not satisfied, the nonparametric Mann-Whitney test was performed. All of the statistical analyses were carried out with Statistica (Statsoft, Tulsa, Oklahoma).

RESULTS

Use of Ex Vivo Tissue Cultures of Human Nasal Polyps. Human nasal polyps obtained from surgical resections and maintained for 24 hours under ex vivo tissue culture conditions generally maintain their histologic organization (Fig 3). Figure 3A,B illustrates the immunohistochemical staining of E-selectin (Fig 3A) and P-selectin (Fig 3B) in 2 pieces of nasal polyp without exposure to budesonide (control conditions). These 2 exemplary illustrations clearly document that in the 2 epithelial compartments of human nasal polyps, E-selectin is expressed to a much greater degree than P-selectin. Figure 3C,D represents the immunohistochemical evidence of the presence of ICAM-1 in the surface epithelium of a control nasal polyp (Fig 3C) and a 250 ng/mL budesonide-treated nasal polyp (Fig 3D). The ICAM-1 staining intensity appears to be stronger in the control condition than in the budesonide-treated condition. Routinely, these histochemical staining patterns were quantitatively determined by means of computer-assisted microscopy as detailed below.

Figure 3E,F presents the glycohistochemical evidence of the presence of binding sites for Le^a in the

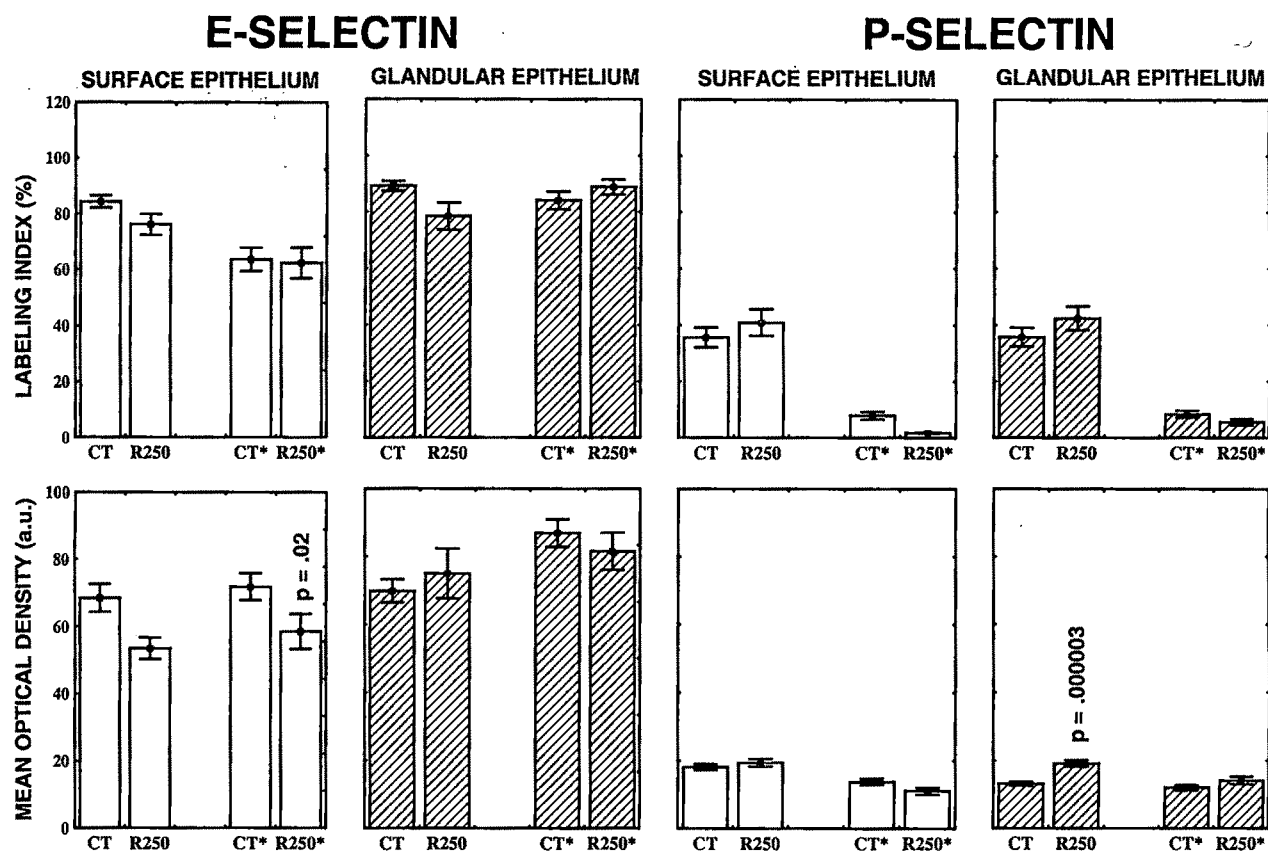


Fig 4. Quantitative evaluation (by means of computer-assisted microscopy) of percentage of immunohistochemically positive tissue area in surface (white bars) and glandular (hatched bars) nasal polyp epithelia (determined by means of labeling index) and staining intensity (determined by means of mean optical density variable) for expression of E-selectin and P-selectin, respectively, in nasal polyp samples treated with concentration of 250 ng/mL (R250, R250*) of budesonide (Rhinocort) or left untreated (CT, CT*). This evaluation was carried out separately for polyps from nonallergic (CT and R250) and allergic (CT* and R250*) patients. Data are illustrated as means (large white or hatched bars) $\pm$ SE (thin black bars).

surface epithelium of a control polyp (Fig 3E) and a 250-ng/mL budesonide-treated (Fig 3F) polyp. The histochemical staining for these binding sites appeared to be less intense in the budesonide-treated condition than in the control one. The Le^a-binding sites were localized at the apical part of the surface epithelium.

All of the budesonide-related data in Figs 4-7 were obtained with the 250-ng/mL budesonide (Rhinocort) concentration. The data for the 2 other tested concentrations (ie, 10 and 50 ng/mL) are, for the sake of clarity, not reported, because they were associated with lower levels of statistical significance than the 250-ng/mL concentration.

Budesonide-Mediated Effects on Immunohistochemical Expression of E- and P-Selectins, ICAM-1, and VCAM-1. Concerning immunohistochemical staining intensity, budesonide induced weaker effects on the levels of P- and E-selectins than on the levels of ICAM-1 and VCAM-1 (Figs 4 and 5). In fact, budesonide slightly decreased the E-selectin concentration (revealed by the MOD variable) in the surface epithelium (the white bars) of the nasal polyps

from the allergic patients (conditions labeled CT* and R250*), and markedly increased the P-selectin concentration in the glandular epithelium (the hatched bars) of the nasal polyps from the nonallergic patients (conditions labeled CT and R250). Budesonide did not significantly ($p > .05$) modify the percentages (revealed by the LI variable) of reactive epithelial cells in either the surface or the glandular epithelium from either the allergic or the nonallergic patients. The basal (control; CT) levels (quantified by means of the LI and the MOD variables) of E-selectin were markedly higher ($p < .001$ to $p < .000001$) than those of the P-selectin, irrespective of the group considered (Fig 4).

Budesonide significantly decreased the percentage of cells immunopositive for ICAM-1 in the glandular epithelium from the nonallergic patients and in the surface epithelium from the allergic patients; it also significantly decreased the ICAM-1 concentration in the surface epithelium from the allergic patients (Fig 5). At the level of VCAM-1 expression, budesonide induced effects identical to those observed for ICAM-1 (Fig 5). In addition, it also sig-

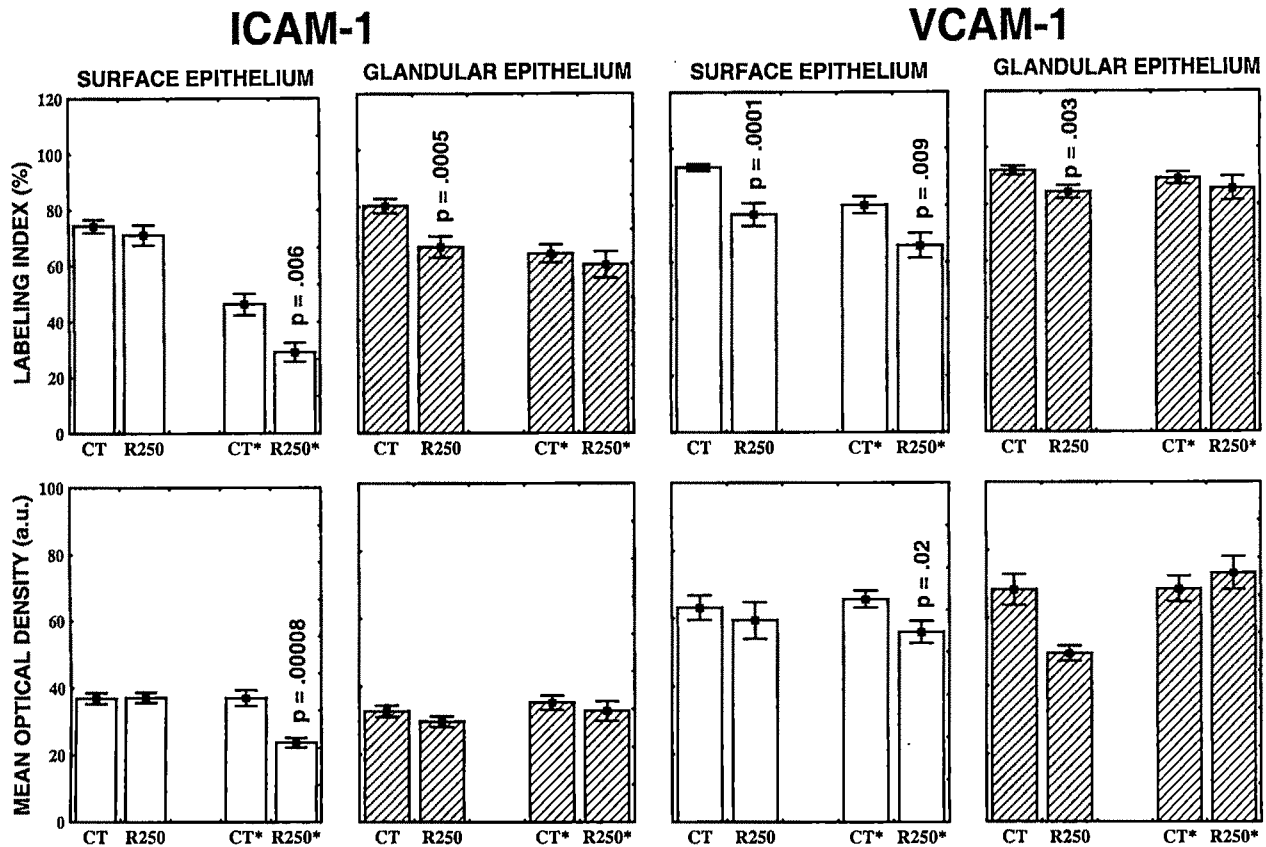


Fig 5. Similarly to Fig 4, this Figure shows quantitative evaluation of expression of ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) in nasal polyp samples treated with concentration of 250 ng/mL of budesonide (Rhinocort) or left untreated. See Fig 4 for further details.

nificantly decreased the percentage of cells immunopositive for VCAM-1 in the surface epithelium of nasal polyps from the nonallergic patients (Fig 5).

Budesonide-Mediated Effects on Immunohistochemical Expression of Le^a and sLe^a Epitopes and Binding Sites Measured Glycohistochemically. Treatment with budesonide did not significantly ($p > .05$) alter either the percentage of cells immunopositive for Le^a or their concentration in the nasal polyp epithelia (Fig 6). In contrast, budesonide exposure led to a significantly decreased Le^a binding site concentration in both epithelial compartments of the nasal polyps from the nonallergic patients and in the surface epithelium of the nasal polyps from the allergic patients (Fig 6). Very similar features were observed with respect to the binding sites for sLe^a, as determined with the labeled neoglycoconjugate (Fig 7). Concerning the sialylated Le^a derivative, budesonide induced a moderate increase in the percentage of cells immunopositive for sLe^a in the surface epithelium of the nasal polyps from the allergic patients (Fig 7).

DISCUSSION

The aim of the present study was the histochemical characterization of effects mediated by budesonide, a glucocorticoid used in clinics, on the levels

of expression of different groups of adhesion molecules in human nasal polyposis, with special emphasis on E- and P-selectins, as well as Le^a epitopes and sialylated derivatives. Human nasal polyps were selected as the study subject because polyps might, in fact, represent a *de novo* inflammatory growth either on the lateral wall of the nose or from the anterior ethmoidal cells.³¹ The predominant inflammatory cells in nasal polyps are eosinophils, to which attention has been turned for analysis of relevant immunologic and inflammatory processes.^{31,32} As stated by Davenpeck et al,³³ eosinophils and neutrophils share the functionality of certain adhesion molecules (ie, P-selectin, E-selectin, and ICAM-1) during the recruitment process, although differences have been described between eosinophil and neutrophil interactions with these molecules.^{33,34}

Concerning cell adhesion molecules, the anti-inflammatory effects of glucocorticoids, including budesonide,³⁵ are already well exemplified by ICAM and VCAM levels.^{10-13,36} Besides these cell adhesion molecules, glucocorticoids also have an influence on selectin expression with supposed relevance for the anti-inflammatory response.^{10-13,36-38} Collectively, the currently available data show a glucocorticoid-induced down-regulation in expression of ICAM,

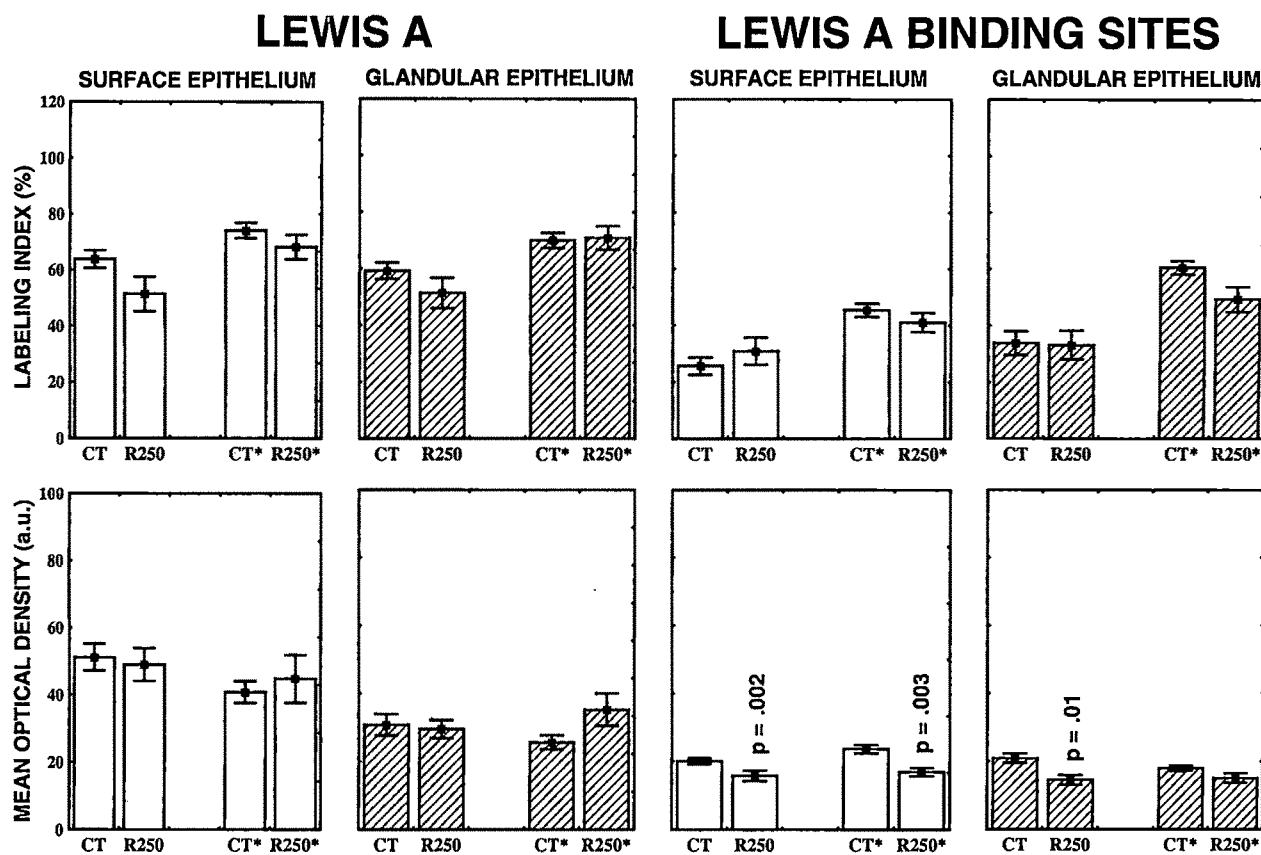


Fig 6. Similarly to Fig 4, this Figure shows quantitative evaluation of expression of Le^a and its binding sites in nasal polyp samples treated with concentration of 250 ng/mL of budesonide (Rhinocort) or left untreated. Expression of Le^a antigens was monitored immunohistochemically by specific antibody, and its binding sites were detected by Le^a-carrying neoglycoconjugate. See Fig 4 for further details.

VCAM, and E-selectin. In contrast, there have been until now no data in the literature on the effects of glucocorticoids on selectin ligand expression, eg, glycoproteins presenting sialylated derivatives of Le^{a/x} epitopes. The data that we obtained in this specific research area with respect to Le^a epitopes are detailed and discussed below.

As a validation of the methods used here, ie, 1) the maintenance for 24 hours under ex vivo organotypical cultures of human nasal polyps obtained from surgery and 2) the quantitative determination by means of computer-assisted microscopy of the histochemical expression of various adhesion molecules in both the absence and the presence of budesonide, we characterized the budesonide-mediated effects on the expression of ICAM-1, VCAM-1, P-selectin, and E-selectin. The data obtained during this validation corroborate those published in the literature. Notably, our data substantiate a budesonide-induced down-regulation of expression of both ICAM-1 and VCAM-1 in both surface and glandular epithelia (Fig 5). Noticeable differences were observed between nasal polyps from allergic and nonallergic patients (Fig 5). A significant budesonide-induced down-regulation of E-selectin expression was observed in the sur-

face epithelium of the nasal polyps from allergic patients (Fig 4). All of these data suggest that part of the anti-inflammatory effect induced by budesonide occurs through a significant decrease of the levels of expression of adhesion molecules whose biological role is to help nasal polyp colonization by inflammatory cells.

We paid particular attention to the epithelial compartments in these nasal polyps, because selectin expression can appear de novo in normal epithelial cells or can be up-regulated in various epithelial cell populations undergoing inflammatory processes. This is the case, for example, for E-selectin expression in epithelial cells of the gingival crevice, the oral epithelium,^{39,40} and the colonic epithelium.^{41,42} The E-selectin expression in these different types of epithelial cells may well be involved in leukocyte trafficking to these epithelial tissue compartments, and this could also be the case for the surface and glandular epithelia in nasal polyps.

Sialyl Le^{a/x} epitopes (Fig 2) form the basis of the recognition unit common to all 3 selectins.^{19,20,43} Studies with mice carrying gene disruptions for selectins and with ligands selective for certain selectins (Fig 1B) point to disparate roles for P-selectin and

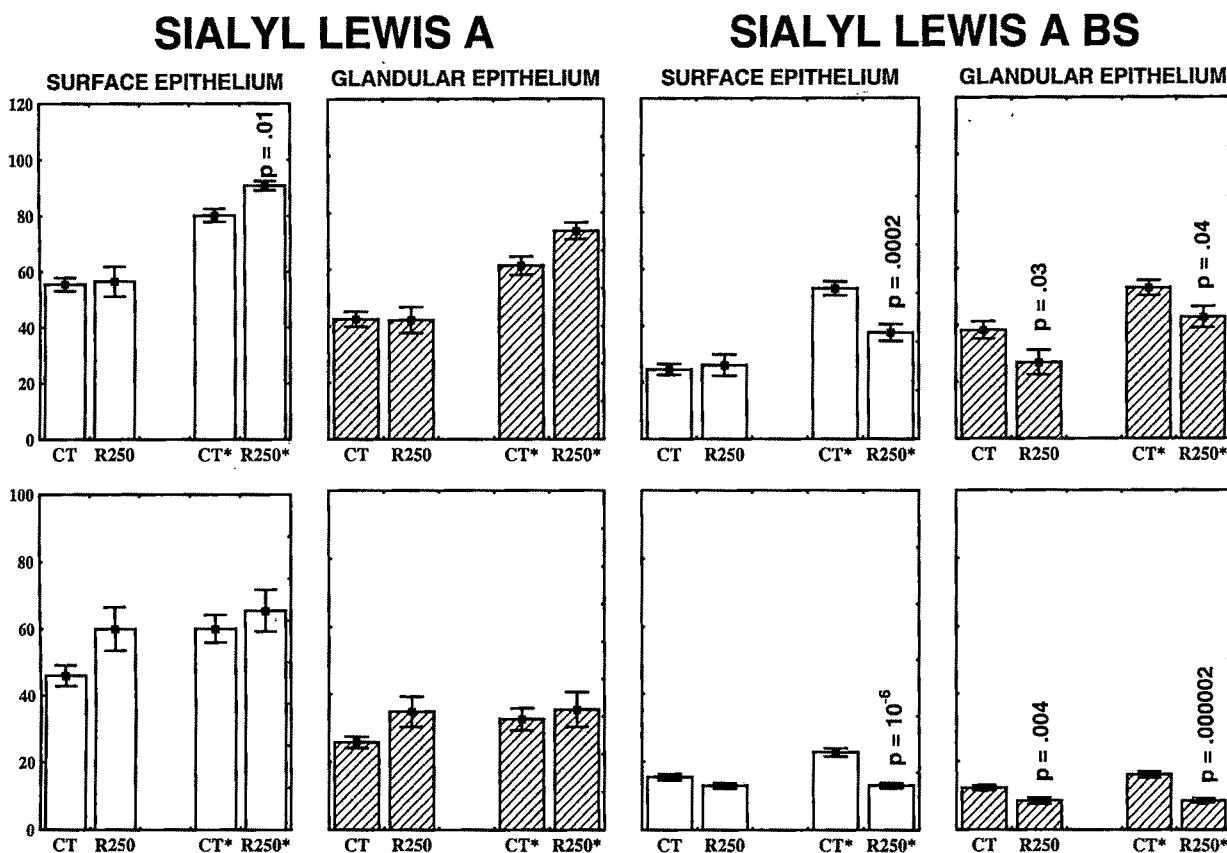


Fig 7. Similarly to Fig 6, this Figure shows quantitative evaluation of expression of sialyl-Le^a (sLe^a) (monitored by specific antibody) and its binding sites (detected by sLe^a-carrying neoglycoconjugate) in nasal polyp samples treated with concentration of 250 ng/mL of budesonide (Rhinocort) or left untreated. See Fig 4 for further details.

E-selectin during in vivo eosinophil and neutrophil recruitment during inflammatory responses.^{43,44} The data in Fig 4 show that E-selectin rather strongly contrasted with P-selectin in both the surface and the glandular epithelia of nasal polyps, whatever the status of the patients (ie, allergic or nonallergic). Our data indicate that budesonide did not dramatically modify the levels of expression of E- and P-selectins (Fig 4), in comparison to what occurred with respect to Le^a and sLe^a binding sites (Figs 6 and 7).

To develop efficient therapies interfering with leukocyte homing to inflamed areas, it will be helpful to delineate how the selective recruitment of leukocyte subtypes during inflammation is orchestrated, as recently reviewed for ischemia/reperfusion states.⁴⁵ An sLe^x glycomimetic TBC1269 has been shown to preferentially inhibit neutrophil adhesion to immobilized P-selectin. With respect to adhesion, eosinophils can adhere more avidly to P-selectin than neutrophils do.^{43,46} This enhanced adhesion of eosinophils to P-selectin is thought to be the result of pronounced surface expression of PSGL-1 (a carrier of distinct target sites for P-selectin and the principal leukocyte counterligand for P-selectin) on eosinophils.⁴⁷ In fact, PSGL-1 has been shown to be the essential ligand for mediating leukocyte rolling on

P-selectin.⁴⁸ Of prime importance for the interaction is the degree to which PSGL-1 harbors adequate glycans for selective binding among the glycoforms. As a tool to probe binding capacity for glycan moieties, we have in this study added neoglycoconjugates to commercial antibodies.

The data obtained for the budesonide-mediated regulation of both Le^a epitopes and their binding sites (Figs 6 and 7) indicate that budesonide markedly reduced the expression of binding sites for Le^a and sLe^a epitopes — a feature that suggests once more a budesonide-mediated anti-inflammatory action mediated through a decrease in the capacity of inflammatory cells to colonize epithelial tissues in nasal polyps.

The way in which structural alterations in related oligosaccharides translate into distinct clinical correlations is also underscored by the role of ABO blood group epitopes and prognostic significance in lung cancer. As demonstrated herein for the Lewis system, the monitoring of specific binding sites for those saccharide epitopes by neoglycoconjugates with a chemically synthesized carbohydrate part has added a new perspective to the way to interpret the occurrence of the glycan determinants, ie, as parts of a

recognition system with signaling potency and medical implications.^{49,50} In fact, presentation of binding sites for A and H (but not B) epitopes by lung carcinoma cells was significantly correlated with prognosis.⁵¹ The inherent specificity of the binding process thus allows us to distinguish between a trisaccharide with a galactose residue carrying an *N*-acetyl group (A; GalNAc) and one without this modification (B; Gal). In further considering the role of generation of a lectin (galectin-1) ligand or lectin presence in 2 cell models — ie, the sialidase-dependent GM1 production on the neuroblastoma cell surface and galectin-1 presence in glioblastoma cells in culture⁵²⁻⁵⁴ — the connection to the ensuing growth control or stimulation of cell migration by this protein-carbohydrate interaction emphasizes the potential of the combined approach presented herein beyond the listed experimental systems.

The consideration of polyps from allergic or non-allergic patients as 2 distinct clinical entities remains

controversial.^{1,2,24} These data indicate that polyps from allergic patients might be biologically different from those of nonallergic patients. Indeed, comparison of the control culture conditions indicates that the levels of expression of the various adhesion molecules and their ligands were significantly different.

In conclusion, it is well established that glucocorticoids, including budesonide, exert their anti-inflammatory effects by regulation of gene expression of effectors and inhibitors of the inflammatory pathway, with various types of cell adhesion molecules serving as current topics of research. The present study documents that such glucocorticoid-induced effects can encompass receptors for Le^a epitopes different from E- and P-selectins on epithelial cells of human nasal polyps. We are pursuing new experiments in order to investigate budesonide-mediated effects on the levels of expression of Le^x/sLe^x epitopes and their binding sites.

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HEARING LOSS IN USHER SYNDROME TYPE II IS NONPROGRESSIVE

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Usher syndrome is an autosomal recessive disorder characterized by sensorineural hearing loss and progressive visual loss secondary to retinitis pigmentosa. In the literature, a possible progression of the moderate to severe hearing loss in Usher syndrome type II (Usher II) is controversial. We studied the development of the hearing loss of 125 patients with a clinical diagnosis of Usher syndrome type II intraindividually and interindividually by repeatedly performing complete audiological and neuro-otologic examinations. Our data show a very characteristic slope of the hearing curve in all Usher II patients and no clinically relevant progression of the hearing loss over up to 17 years. The subjective impression of a deterioration of the communicative abilities of Usher II patients must therefore be attributed to the progressive visual loss. The patients should be reassured that changes in their hearing abilities are unlikely and should be provided with optimally fitted modern hearing aids.

KEY WORDS — hearing loss, Usher syndrome type II.

INTRODUCTION

Usher syndrome is an inherited disorder that clinically presents with different degrees of sensorineural hearing loss and progressive visual loss caused by retinitis pigmentosa. The pattern of inheritance is autosomal recessive. There are 3 types of Usher syndrome distinguished clinically, and different subtypes. Patients with type I Usher syndrome (Usher I) are born with profound sensorineural hearing loss; the vestibular organs can either be normal (cochlear form of Usher I, called cUSH1) or defective (vestibulocochlear form of Usher I, called vcUSH1).¹ Moderate to severe sensorineural hearing loss and regularly functioning vestibular organs characterize Usher syndrome type II (Usher II). Usher syndrome type III (Usher III) was originally reported in Finland and presents with progressive hearing loss. The retinitis pigmentosa is believed to be comparable in all types. The prevalence of Usher syndrome was estimated as between 3.3 and 4.4 per 100,000 persons. More recent estimates support the impression of an even higher prevalence of up to 8 per 100,000 persons.

The visual impairment due to retinitis pigmentosa increases from a mildly disabling loss of the visual field at the onset of clinical symptoms to only residual vision in the center of the retina in fully developed retinitis pigmentosa. Progression of hearing loss in Usher type I cannot be expected, as Usher I patients have no functional hearing available.

Progression of the hearing loss in Usher II is con-

troversially discussed in the recent literature. Kimberling and Möller² described no audiological measurable deterioration of the hearing abilities over decades. Van Aarem et al³ saw a great variability in the hearing acuity as measured by pure tone audiograms of Usher II patients, but found two major patterns of hearing curves: one stationary and one rapidly progressive as defined by an annual threshold change of more than 1 dB. Usher III was genetically excluded from this study; all patients belonged to the genetic Usher IIa subtype as defined by linkage. Wagenaar et al,⁴ of the same Dutch group, saw an age-related hearing loss that was not explainable by presbycusis alone. The authors described an average annual threshold increase of 0.7 dB, which was similar at all frequencies studied. In Usher III, progression of the hearing loss has been described as a typical clinical feature. In addition, some typical ophthalmologic findings have been significantly related to Usher III.⁵ Usher III was originally believed to be geographically restricted to Finland and to Finnish immigrants in the southern United States, probably because of a founder effect. However, it has now been found in several persons of varied European ancestry⁶ (also Kimberling, unpublished observations).

In the present study, we examined the hearing curves of 125 patients with clinically diagnosed Usher II of the Heidelberg Usher collective to evaluate the development of the hearing loss in relation to the age of the patients and in individual patients over a longer time period of up to 17 years.

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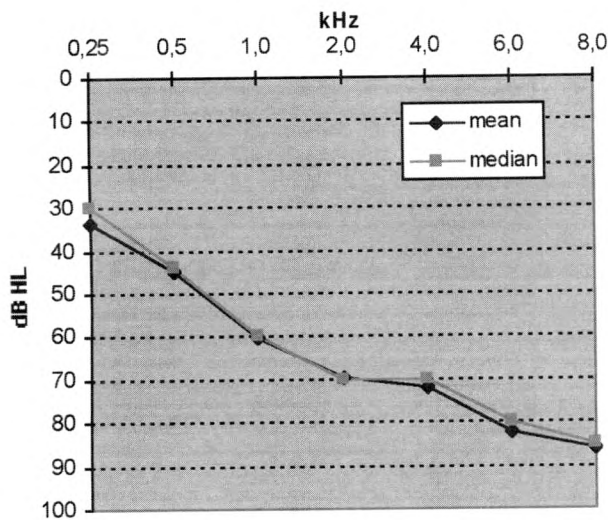


Fig 1. Mean and median of hearing loss in Usher II patients (n = 125).

METHODS

Otoneurologic and ophthalmologic studies were conducted on 125 Usher II patients of 115 families of the Heidelberg Usher collective ranging between 14 and 72 years of age. The clinical course of the hearing loss, as well as the ophthalmologic status and the course of the retinitis pigmentosa, defined Usher type II patients. Usher III was excluded clinically, as the hearing loss was stable in all examined patients and the ophthalmologic examination revealed no sign of Usher type III such as clinically significant hypermetropia or astigmatism.⁵ All patients were given ophthalmologic examinations in the Department of Ophthalmology of the University of Heidelberg. All patients presented with different degrees of retinitis pigmentosa. The otoneurologic examinations were performed in the Department of Otolaryngology–Head and Neck Surgery of the University of Heidelberg. Standard history-taking and general otolaryngological examination were performed. All patients were given audiological and neuro-otological examinations including pure tone audiometry, tympanometry, stapedius reflex audiometry, measurement of transitory evoked otoacoustic emissions, videonystagmography (including caloric stimulation with hot [44°C] and cold [30°C] water), and posturography. In addition, brain stem evoked audiometry was performed in 71 patients to rule out a retrocochlear cause of hearing loss.

The examinations were repeatedly performed between 1990 and 1999. In 56 cases, earlier test results were obtained from other institutions dating back as far as 17 years. The results were set in relation to the age of the individual patients. To differentiate among low, middle, and high frequencies, 3 intervals were defined: respectively, 0.5 to 1 kHz, 2 to 4 kHz, and 6 to 8 kHz.

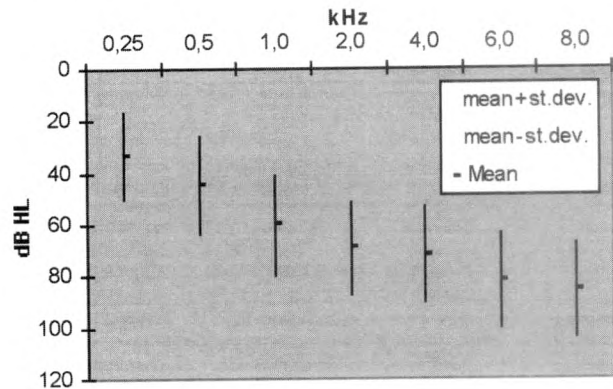


Fig 2. Mean of hearing loss in Usher II patients $\pm$ 1 SD (n = 125).

RESULTS

Pure tone audiometry revealed in all 125 patients a moderate to severe sensorineural hearing loss that had been present since birth. The mean age ($\pm$ SD) of the Usher II patients was 39.7 ± 14.6 years (range, 14 to 72 years).

None of the patients complained of any form of tinnitus. The hearing curves varied considerably and showed in all patients a typical slope toward the high frequencies (Figs 1 and 2). Tympanometry revealed normal results in accordance with the clinically normal eardrum and middle ear anatomy. Stapedius reflex audiometry and transitory evoked otoacoustic emissions, as well as brain stem evoked audiometry, ruled out a retrocochlear cause of hearing loss in accordance with the cochlear defect in Usher syndrome.⁷ Videonystagmography and posturography showed normal function of the peripheral vestibular organs in all Usher II patients.

Audiograms were repeatedly obtained and reviewed, and there was no intraindividual decrease in hearing function, although some individuals reported that their subjective feeling of hearing ability had decreased. This was also the case in those audiograms that were obtained within an interval of up to 17 years, providing a longitudinal comparison of the hearing curves.

A linear regression analysis of hearing against age was performed for each of the 3 pooled frequency data sets: low, middle, and high. No significant correlation between age and degree of hearing loss was observed. Specifically, the analysis of hearing loss versus age for the low frequencies resulted in a slope of 0.125 with a coefficient of correlation of 0.09 (Fig 3). For the middle frequencies, the regression coefficient was 0.22 and the coefficient of correlation was 0.18 (Fig 4). The high-frequency regression coefficient was 0.13, and the coefficient of correlation was 0.095 (Fig 5).

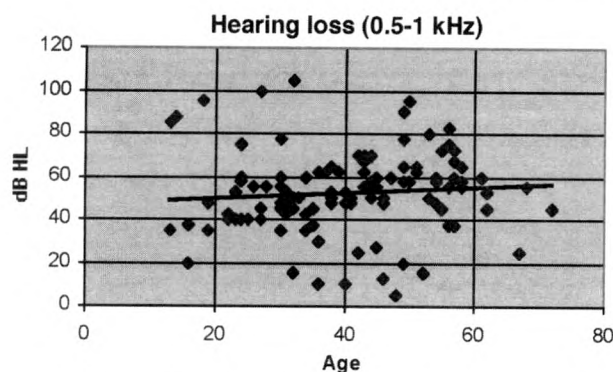


Fig 3. Correlation between age and hearing loss in low-frequency range of 0.5 to 1 kHz.

DISCUSSION

Usher syndrome type II is widely accepted as a clinically and genetically heterogeneous autosomal recessive disorder that combines various degrees of sensorineural hearing loss with progressive retinitis pigmentosa. There is a sloping hearing curve with a mild hearing loss in the low frequencies, increasing to a severe to profound hearing loss in the high frequencies. Usher II patients usually benefit from hearing aids.

It is still arguable whether the hearing loss in Usher type II is progressive or not. This would be an important way to distinguish Usher III from Usher II, as Usher III is characterized by progressive hearing loss. The ophthalmologic distinction between Usher II and Usher III can be performed clinically, as in Usher III the course of the visual handicap (clinically significant hypermetropia) and the typical refractive errors (astigmatism) are pathognomonic for Usher III.⁵

Most Usher II patients are aware that their vision loss is progressive and that ultimately their visual capabilities will be severely compromised. Their hearing ability therefore becomes even more precious to them, and they become understandably concerned

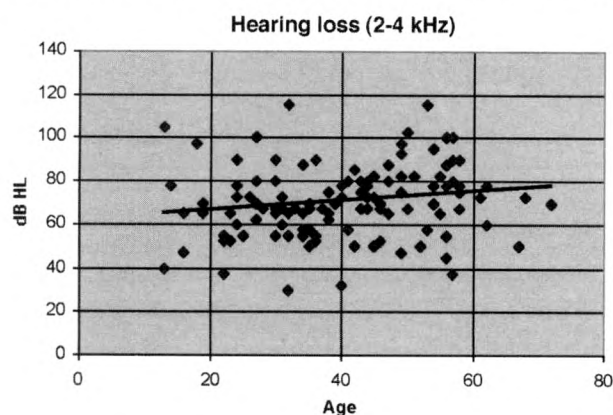


Fig 4. Correlation between age and hearing loss in middle-frequency range of 2 to 4 kHz.

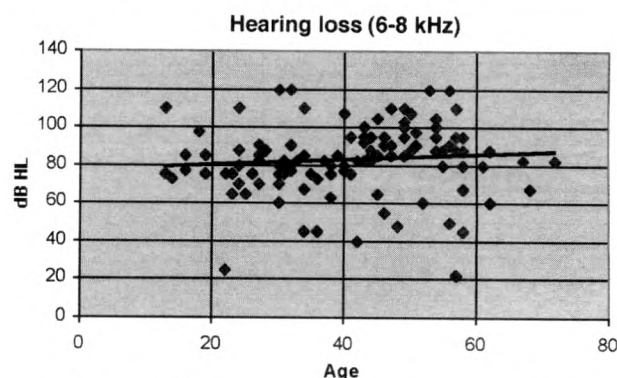


Fig 5. Correlation between age and hearing loss in high-frequency range of 6 to 8 kHz.

about any added decrease in their hearing capabilities. To answer questions on this topic from their patients, consulted physicians have to be aware of potential changes in the hearing abilities of Usher II patients.

Many Usher II patients have the clinical impression that their hearing has been deteriorating over the years. While there may be deterioration in a few cases, it seems likely that this impression is due to the decrease in visual ability that reduces the patients' ability to supplement auditory information with visual cues. It is important to reassure such patients so that they should not have the mistaken impression that they are destined to become both deaf and blind.

Our data show that if there is any progression to the hearing loss in Usher type II, such progression is slow — probably much less than 1 dB per year. In consequence, the physician should be aware that any change in the hearing status of the typical Usher II patient may signal some other disorder that may be treatable.

It has therefore been the goal of our study to determine the hearing abilities of the Usher II patients of the Heidelberg Usher syndrome group in comparison within the group and within each patient when former audiograms were available. The hearing curves of 125 patients with clinically diagnosed Usher II syndrome were evaluated in relation to the patient's age. In 56 of the Usher II patients, former audiograms were available and could be analyzed for intraindividual changes of the hearing curves.

In our patient group, there was a great variability of hearing loss documented in the hearing curves. Nevertheless, the hearing loss followed the same pattern in all audiograms. We confirm the findings of Kimberling and Möller² that a sloping hearing curve is very characteristic of Usher II. We stress that the diagnosis of Usher II should be reconsidered if the hearing curve differs from the typical pattern without an obvious reason.²

The most interesting result of our study was that we found no sign of progression of hearing loss in the 125 patients with Usher II. This was seen both in patients for whom several audiograms were obtained within the study time (1990 to 1999) and in 56 patients for whom former audiograms from up to 17 years before were available from other institutions. This lack of progression stands in contradiction to the findings of van Aarem et al³ and Wagenaar et al.⁴ That Dutch group saw an annual threshold increase greater than could be explained by presbycusis alone. They studied genetically identified Usher IIa cases, whereas we primarily studied clinically diagnosed Usher II cases. The differences between the studies might be due to a varied contribution to the sample from other subtypes of Usher II, namely, Usher IIb and Usher IIc. An alternative explanation for the difference might be innate population differences or differences in the treatment of the hearing losses. The Dutch population may also have a higher frequency of certain genes that may perhaps exacerbate the effect of the USH2A mutant gene. Kimberling and Möller² also found no significant progression of the hearing abilities in Usher II patients over decades. This patient group was also probably varied with respect to its composition with regard to the Usher II subtype.

Genetic analysis of the patient group discussed in this study is currently in progress (Kimberling, unpublished observations). It will be interesting to correlate our findings with the genotypes and to further compare our results with the findings of Wagenaar et al⁴ in 26 genetically identified Usher IIa cases. It would be very unlikely that our group of 125 Usher II patients would not include Usher IIa patients, since Usher IIa accounts for a majority of Usher II patients.

In our study group, a number of Usher II patients complained of the feeling of a hearing deterioration over the years. It might have been due to the fact that Usher II patients are born with a hearing deficit and are used to supplementing their hearing function by lipreading. As the visual function deteriorates, the subjective impression of acoustic communication deteriorates, too, and the patients attribute this to a progression of their hearing loss. In those patients who complained about a progression of hearing loss, we found in no case a measurable change of the hearing curve. Therefore, the consulted otologist can reassure his or her Usher II patients that changes in their hearing abilities are unlikely and should emphasize the continuous verification of an optimal provision with modern hearing aids.

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OSTEOCARTILAGINOUS DIFFERENTIATION OF MUCOSAL MELANOMA IN THE SINONASAL CAVITY

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We present a very rare case of mucosal melanoma with osteocartilaginous differentiation (MMOD). This is the first report of MMOD originating in the sinonasal cavity. Preoperative diagnosis of this tumor from specimens resected for biopsy alone is very difficult, because it has 2 histopathologic components: a primary melanoma site and the part of the melanoma with osteocartilaginous differentiation. The immunohistochemical technique is useful in histopathologic diagnosis of this tumor. Malignant melanoma usually shows reactivity with MelanA (melanoma tumor antigen) and S-100 proteins. We diagnosed this case as MMOD because both regions in this tumor were stained with MelanA and S-100 proteins. Magnetic resonance imaging provides the best clinical ability to detect malignant melanoma in the sinonasal region. In this case, the melanotic melanoma site in the posterior ethmoid sinus appeared hyperintense to gray matter on T1-weighted image magnetic resonance imaging. Thus, it would have been possible to diagnose this case as MMOD before operation, even though we could not obtain a biopsy specimen from the posterior ethmoid sinus lesion.

KEY WORDS — mucosal melanoma, osteocartilaginous differentiation, sinonasal cavity.

INTRODUCTION

Mucosal melanoma with osteocartilaginous differentiation (MMOD) of the nasal cavity and paranasal sinus is an uncommon tumor. Banerjee et al¹ reported that 18 cases of MMOD in the whole body had been reported. The report of Hoorweg et al² was the first and only report to describe MMOD of the nose and paranasal sinus. However, theirs was a recurrent case of melanoma in the sinonasal region. Mucosal melanoma is a rare tumor in the nasal cavity and paranasal sinuses. Melanomas of the sinonasal cavity constitute fewer than 4% of all sinonasal neoplasms and account for approximately 1% of all malignant melanomas.³ It is difficult to differentiate a melanoma from other tumors by histopathologic examination of only biopsy specimens. Some melanomas have been misdiagnosed before operation as other tumors, such as undifferentiated carcinoma, olfactory neuroblastoma, rhabdomyosarcoma, and malignant lymphoma.⁴ Two histopathologic components are included in the MMOD: the primary melanoma site and the part of the melanoma with osteocartilaginous differentiation.¹ The histopathologic diagnosis of MMOD is very difficult if the tumor specimen is resected only from the portion with cartilage and bone formation. Magnetic resonance imaging (MRI) is a useful tool for preoperative diagnosis of melanoma. In general, melanoma has a high signal intensity on T1-weighted images and a low signal intensity on T2-weighted images as compared to gray matter.⁵ Herein, we describe the first primary case of a patient with MMOD

that was initially diagnosed before operation as mesenchymal chondrosarcoma.

CASE REPORT

A 75-year-old woman presented to the otorhinolaryngology clinic of Kanazawa University Hospital on November 27, 2000, with massive epistaxis from the left nostril that had recurred for about 8 months. Endoscopic examination revealed a dark violet polypoid tumor arising from the olfactory cleft in her left nasal cavity and a gray mass continuing from the nasal tumor into the nasopharynx. An MRI scan was performed. An axial T1-weighted image showed a lesion that was high-intensity to gray matter in the posterior ethmoid sinus, bordered by a very high-intensity area that appeared hemorrhagic (Fig 1A). A corresponding axial T2-weighted image showed a lesion that was low-intensity to gray matter, bordered by very low signal intensity (Fig 1B). A coronal T1-weighted image through the nasal cavity showed a lesion that was low-intensity to gray matter and continuous with the high-intensity lesion in the posterior ethmoid sinus that was adjacent to the cranial base (Fig 1C). An axial T2-weighted image showed a lesion that was intermediate-intensity to gray matter and continuous with the low-intensity lesion in the posterior ethmoid sinus (Fig 1D). The very high-intensity area in the left maxillary sinus appeared to be due to an obstructed secretion. On histopathologic examination of a biopsy specimen from the nasal polypoid lesion, the tumor was found to be a mesenchy-

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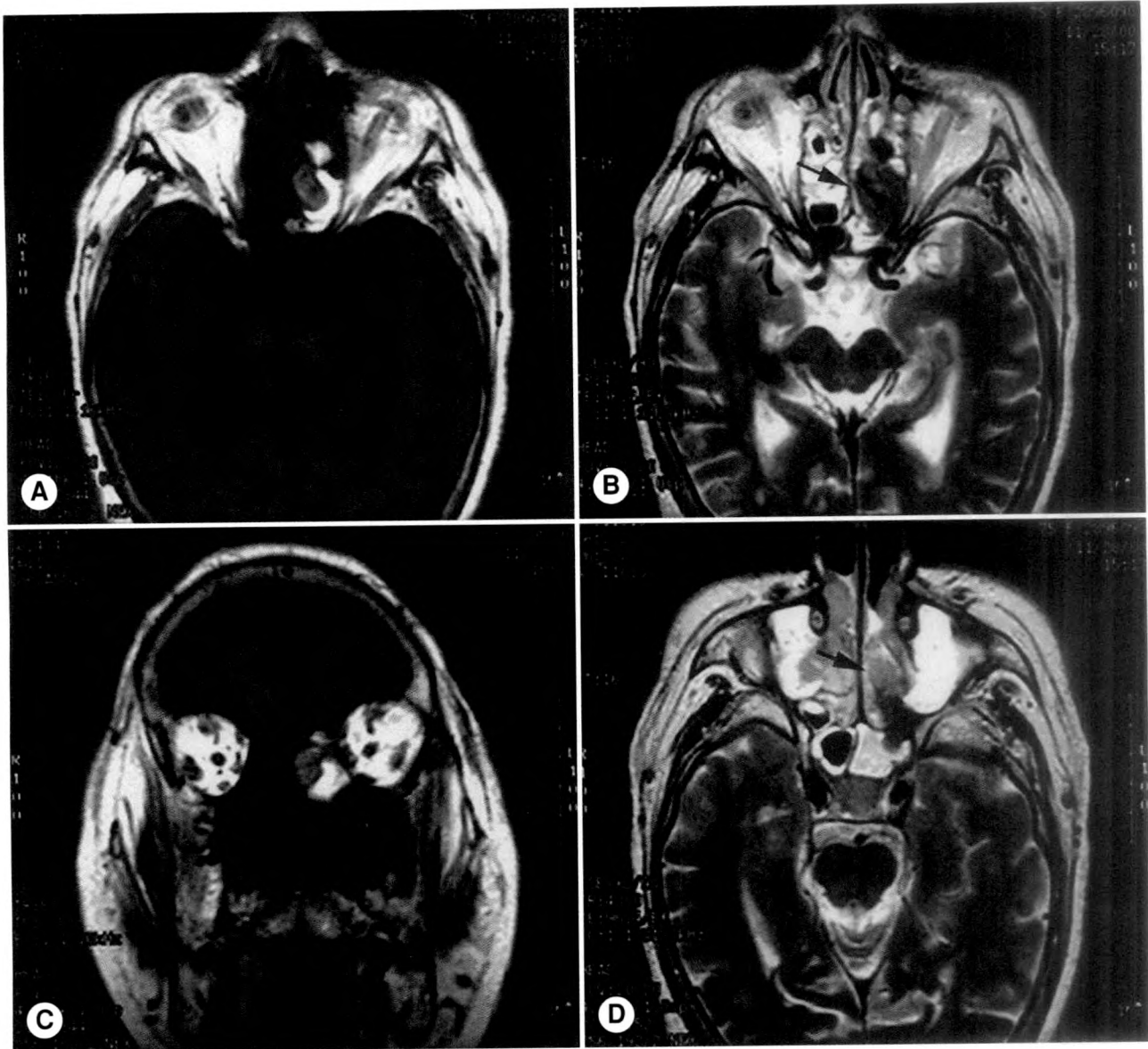


Fig 1. Magnetic resonance imaging. **A)** Axial T1-weighted image shows that tumor in posterior ethmoid sinus has high signal intensity to gray matter (arrow) and is bordered by very high-intensity area that appears hemorrhagic. **B)** Corresponding axial T2-weighted image shows lesion that is low-intensity to gray matter (arrow) and is surrounded by very low-intensity area in posterior ethmoid sinus. **C)** Coronal T1-weighted image shows lesion that is low-intensity to gray matter in nasal cavity (arrow) and is continuous with high signal intensity in posterior ethmoid sinus. **D)** Axial T2-weighted image shows lesion that is intermediate- or high-intensity to gray matter in nasal cavity (arrow) and is continuous with low signal intensity in posterior ethmoid sinus.

mal chondrosarcoma that was extremely undifferentiated. Surgical treatment was not considered because of the many complications of the patient's hypertension, diabetes, hyperlipidemia, herniation of a lumbar disc, and arrhythmia and the lack of histopathologic differentiation of the tumor. The patient was treated by intra-arterial chemotherapy with cis-diaminedichloroplatinum (CDDP) and radiotherapy (30 Gy to the nose and paranasal sinuses). First, 100 mg of CDDP was superselectively administered through the feeding artery (the left maxillary artery) every 3 weeks. Although the tumor became gradually reduced in the nasopharynx, there was no regression of tumor

size in the nasal cavity and paranasal sinuses. The patient subsequently underwent an excision by left lateral rhinotomy under general anesthesia. The macroscopic findings of the tumor were a dark violet polypoid tumor in the nasal cavity and a black polypoid tumor continuous with it in the left posterior ethmoid sinus. The postoperative histopathologic examination revealed that the tumor in the left posterior ethmoid sinus was composed of epithelioid cells with large round nuclei and massive melanin pigments (Fig 2A). The tumor in the left nasal cavity showed the proliferation of short spindle cells and cartilaginous cells (Fig 2B). Since an immunohistochemical study re-

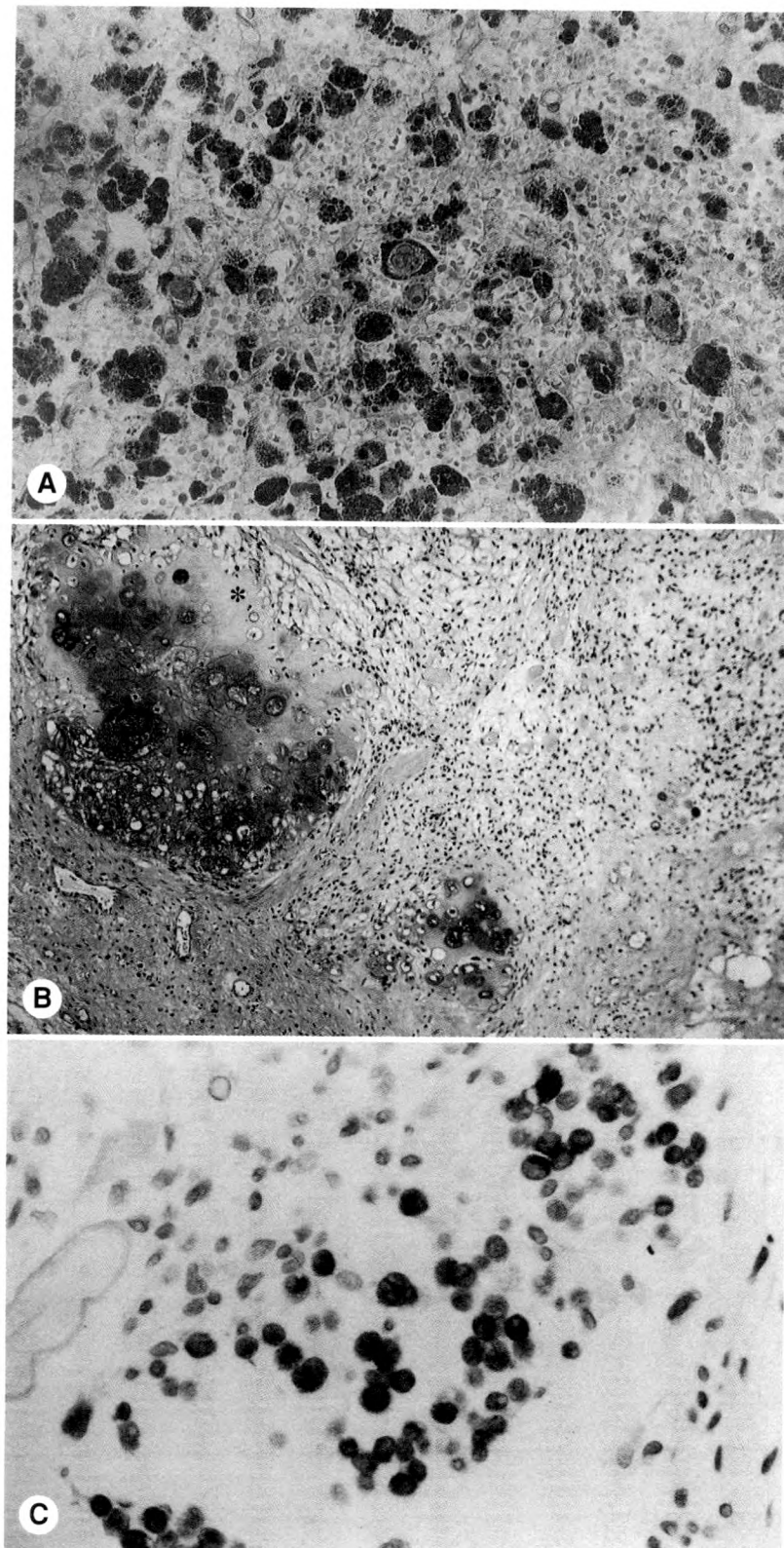


Fig 2. Histologic photomicrographs. **A)** Posterior ethmoid sinus tumor shows many invasive atypical cells containing massive melanin granules in cytoplasm (H & E, original $\times 400$). **B)** Nasal tumor has 2 histologic components (H & E, original $\times 100$). One is area of small spindle cells in myxoid stroma, and other is area of cartilaginous matrix with atypical cartilaginous cells (asterisk). **C)** Small spindle cells of nasal tumor are diffusely positive for MelanA protein in cytoplasm (original $\times 600$).

vealed positive staining of the neoplastic cells with S-100 and MelanA (melanoma tumor antigen; Fig 2C) in both regions, this tumor was diagnosed as an MMOD. Although combination chemotherapy was suggested, consisting of dimethyltriazeno imidazole

carboxamide, amino methyl pyrimidinyl chlorethy nitrosourea hydrochloride, vincristine, and CDDP, the patient rejected this treatment. She has been reviewed regularly and was disease-free at 14 months after treatment.

DISCUSSION

An MMOD arising in the sinonasal region is an extremely rare tumor. According to previous studies, only 18 cases have been reported.¹ Only 1 case of MMOD occurring in a neck lymph node with melanoma in the sinonasal cavity was reported by Hoorweg et al.² To the best of our knowledge, this is the first report of a primary MMOD in the sinonasal cavity. A possible explanation of the cartilaginous formation with melanoma has been suggested by Lucas et al.⁶ They wrote that melanomas were mesenchymal tumors and suggested that mesenchymal metaplasia might result in the production of osteocartilaginous components. It is known that mesenchymal tumors originating from the neural crest have the ability to form stroma, bone, and cartilage.²

The differential diagnosis of MMOD in the nose and paranasal sinus includes sarcomatoid squamous cell carcinoma, osteogenic sarcoma, mesenchymal chondrosarcoma, and malignant change in a pleomorphic adenoma.¹ We diagnosed this tumor as mesenchymal chondrosarcoma at the initial diagnosis from histopathologic examination of a small biopsy specimen. Mesenchymal chondrosarcoma usually displays small, round, undifferentiated cells in a hemangiopericytoma-like pattern interspersed with islands of cartilage.⁷ Prominent spindle cell areas may also be present with a consequent increased diagnostic difficulty. The expression of MelanA and S-100 proteins is characteristic of melanomas.⁸ Evans et al.⁸ reported that melanocytes were more clearly stained with MelanA than with S-100 protein. Although the diagnosis of MMOD is very difficult to make by histopathology, immunohistochemical examination is

useful for definitive diagnosis of MMOD.

Although melanoma has a poor prognosis, early detection, diagnosis, and treatment of this disease is beneficial for longer patient survival. When the imaging findings can suggest the pathological nature of the lesion, delays in diagnosis and treatment can be avoided. The best imaging tool for diagnosis of a sinonasal mass is MRI. It was revealed by previous studies using MRI that melanotic melanoma showed hyperintensity to gray matter on T1-weighted images and low to intermediate signal intensity to gray matter on T2-weighted images.⁵ This finding is generally suggested to be due to the paramagnetic properties of melanin,⁹ although some studies suggest that these findings in MRI are due to free radical formation in melanin.¹⁰ In any case, the MRI intensity of melanoma relates to the melanin content of the tumor. Amelanotic melanoma in the nasal cavity shows intermediate signal intensity to gray matter on T1-weighted images.⁵ The posterior ethmoid lesion that was diagnosed as melanotic melanoma on histopathology showed high signal intensity to gray matter on T1-weighted MRI. However, the tumor of the nasal cavity that was diagnosed as the portion with cartilaginous differentiation in the melanoma presented a low signal intensity to gray matter on T1-weighted MRI. This finding suggests that it was possible to diagnose this case as MMOD rather than as mesenchymal chondrosarcoma before operation, because the high intensity to gray matter of the T1-weighted MRI in the posterior ethmoid sinus lesion indicated this tumor to be a melanotic melanoma, and we could not obtain a biopsy specimen from the posterior ethmoid sinus lesion.

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HYPERVISCOELASTICITY OF EPIPHARYNGEAL MUCUS MAY INDUCE GLOBUS PHARYNGIS

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Detailed observation reveals a lump of translucent sticky mucus standing in the epipharynx of most globus patients, suggesting that the mucus adherent to the epipharynx causes a globus sensation. The epipharyngeal mucus of 70 consecutive globus patients was sampled via the nasal cavity. Fucose and sialic acid, the determinants of the viscoelasticity of mucus, were measured. Twenty-three patients were also asked to complete the Throat Questionnaire so we could evaluate the correlation between the degree of globus sensation and the viscoelasticity of the mucus. The mucus of 10 healthy subjects was sampled, analyzed, and compared with the data of the patients. The fucose and sialic acid concentrations found in the patients with globus pharyngis were significantly higher than those in the control subjects (Mann-Whitney U test, $p < .01$). The fucose concentrations correlated significantly with the globus pharyngis scores (Spearman correlation, $p < .05$). We conclude that there is a close association between stagnant mucus in the epipharynx and globus sensation.

KEY WORDS — epipharynx, globus sensation, mucus, viscoelasticity.

INTRODUCTION

Globus pharyngis is defined as the sensation of having a lump in the throat. As, in many cases, routine otolaryngological clinical examination fails to reveal the underlying pathogenesis, it has been widely regarded as being of psychological origin.

More recently, globus pharyngis has been reported to be due to esophageal disorders, such as gastroesophageal reflux disease (GERD), motility disorders, and structural abnormalities.¹ That idea may, however, be caused by the high prevalence of concomitant asymptomatic GERD in the healthy Western population.¹ Wilson et al² showed that there was a similar rate of reflux in the control group in their study and insisted that abnormal exposure of the esophageal mucosa to acid and distal esophageal dysmotility were not the etiologic factors in the majority of patients with globus pharyngis. In light of our personal experience as otolaryngologists, we also think that GERD may be present in the minority of patients, while in the majority, some other physical basis for the disorder exists.

Sensory denervation of the central palate following laser-assisted uvulopalatoplasty can be a cause of globus pharyngis.³ The degree of this unpleasant globus sensation seems to be related to the size of the insensible area left by uvulopalatoplasty.³ It is probable that some patients with globus pharyngis present an organic cause of this disorder in the phar-

ynx. Detailed evaluation of the epipharynx reveals a lump of colorless translucent mucus standing in the epipharynx of most patients with globus pharyngis (Fig 1), but not in healthy subjects. We hypothesized that there might be a lump of mucus of high viscosity and spinability stagnant in the epipharynx in spite of swallowing and mucociliary transport that causes the globus sensation.

The viscosity and the elasticity of mucus are im-

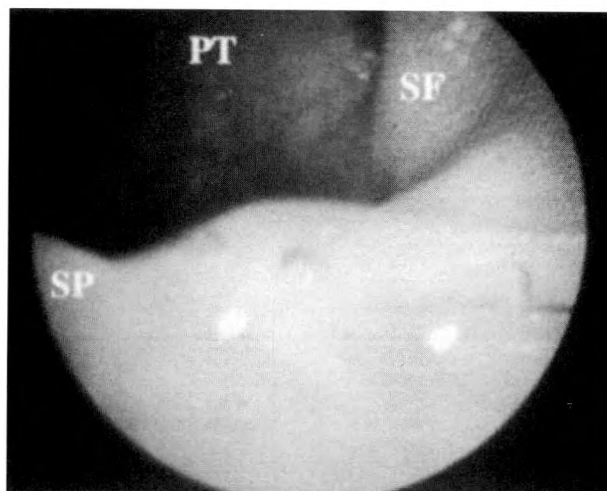


Fig 1. Epipharynx of patient with globus pharyngis as observed under flexible fiberscope. Colorless, translucent mucus can be observed on posterior-superior aspect of soft palate (SP). PT — pharyngeal tonsil; SF — salpingopharyngeal fold.

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TWELVE-ITEM THROAT QUESTIONNAIRE

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1. Feeling of something stuck in throat
 2. Pain in throat
 3. Discomfort in throat
 4. Difficulty in swallowing food
 5. Dryness of throat
 6. Indigestion
 7. Hoarseness
 8. Catarrh down throat
 9. Throat closing off
 10. Have to keep swallowing
 11. At present, how annoying do you find your throat?
Not at all Almost not Moderate Severe
Unbearable
 12. How often are you aware of your throat?
Not at all Almost never Once a month Once a week
Once/twice a week Daily All the time
- Items 1 through 10 are scored on 5-point scale:
None Mild Moderate Severe Unbearable
None = 0, Unbearable = 4
- Items 1-5 and 9-11 are combined to give "total throat score" with maximum value of 32.
-

portant determinants of mucociliary transport.⁴ The concentrations of fucose and sialic acid positively correlate with the viscoelasticity of nasal mucus.⁴ To investigate the organic disorder eliciting the globus sensation, we examined the concentrations of fucose and sialic acid in epipharyngeal mucus.

METHODS

The subjects were 70 consecutive patients (27 men and 43 women, 23 to 87 years of age) who visited our clinic complaining of a globus sensation of unknown origin in the throat between October 1, 1999, and January 31, 2001. A routine diagnostic workup including a patient history, otolaryngological examination, and flexible endoscopy of the hypopharynx and cervical esophagus failed to reveal the cause of the disorder.

Epipharyngeal mucus was collected by aspiration with a thin pipe of 1 mm in outer diameter (Nagashima Medical Instruments Co, Ltd, Tokyo, Japan) via the nasal cavity under the control of a fine flexible fibroscope (ENF Type XP, Olympus, Tokyo). Care was taken to avoid stimulating the mucosal surface during the collection. To examine the reproducibility of the data, we also collected mucus 2 weeks after the first sampling in 4 patients who still complained of a globus sensation in spite of medication (Ambroxol, 45 mg/d for 14 days). Nasal smears for eosinophils obtained from the sampled mucus were studied with May-Grünwald-Giemsa staining to examine the possibility of an allergic reaction. Twenty-

three of the patients (10 men and 13 women, 23 to 78 years) who visited us between September 1, 2000, and January 31, 2001, were asked to complete the Throat Questionnaire⁵ (see Table), which yielded a current globus pharyngis score, a total throat score (TTS; derived from 8 items relating to pharyngeal aspects of globus sensation), and a frequency-of-throat-awareness score on the day of mucus sampling.

Ten healthy volunteers (7 men and 3 women, 23 to 56 years of age) took part in the study as controls. As a measurable amount of secretion could not be obtained from the healthy subjects, the nasal mucosa was provoked with acetylcholine in metered dose pump sprays (Novartis, Basel, Switzerland) that delivered approximately 0.09 mL per puff.⁶ One puff of acetylcholine (4.5 mg, Daiichi, Tokyo) was administered into each nostril. Shortly after the dosing, excess fluid in the nostril was gently wiped away with gauze. Epipharyngeal secretions were sampled 5 minutes after the provocation by aspiration in the same manner as in patients.

The collected samples were stored at -40°C and were preserved for the biochemical measurements. Before use, the samples were left at room temperature for 1 hour. Each nasal sample was weighed and mixed with 19 volumes of distilled water, and the mixture was homogenized in an ice bath. Fucose was measured by the cysteine sulfate method described by Winzler,⁶ and sialic acid by an enzymatic assay.⁷

The concentrations of fucose and sialic acid in samples from patients with globus pharyngis were compared with those of normal volunteers by non-parametric analysis (Mann-Whitney U test) to reduce the influence of any outliers. The association between the concentrations of fucose and sialic acid from globus patients and the TTS, which was associated with the severity of globus symptoms,⁵ was examined by Spearman rank correlation. A *p* value of less than .05 was considered statistically significant. Informed consent was obtained from all of the subjects.

RESULTS

The amounts of collected epipharyngeal secretion in patients with globus pharyngis and controls were 0.175 ± 0.015 mL (mean $\pm$ SE; median, 0.157; range, 0.011 to 0.560) and 0.317 ± 0.047 mL (median, 0.292; range, 0.124 to 0.589), respectively. In both groups, the sampled secretions were colorless and translucent; however, those from patients were mucinoid, while those from controls were mucoserous. Of the nasal smears of the sampled mucus, 7.5% (3/40 patients) were positive for the presence of eosinophils ($\geq 4\%$).

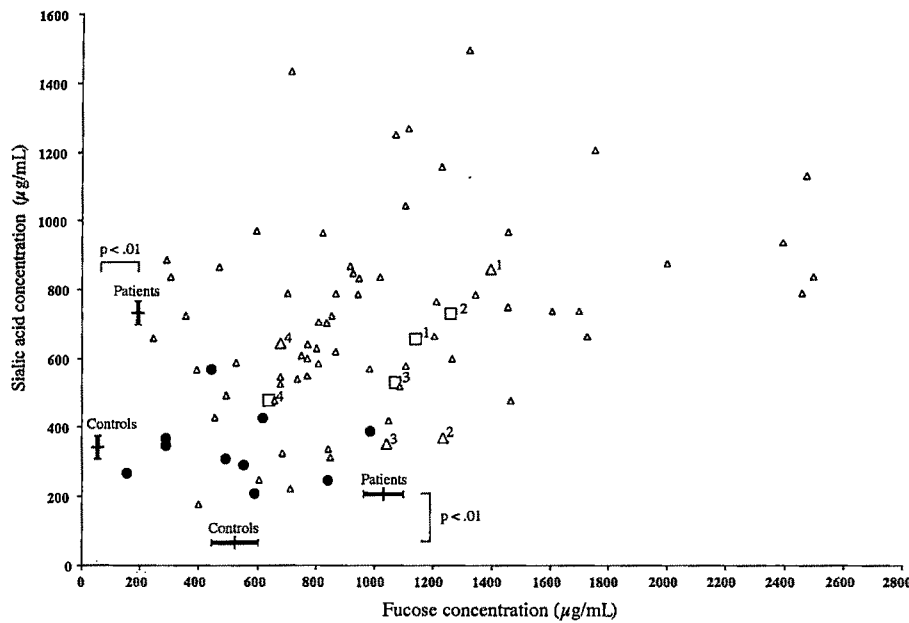


Fig 2. Fucose concentrations (horizontal) and sialic acid concentrations (vertical) in epipharyngeal secretions (mean $\pm$ SE). Open triangles show patients with globus sensation, and filled circles show controls. Open squares indicate data obtained 2 weeks after first sampling in patients with sustained globus sensation. (Number on right shoulder of open triangle and open square indicates data from same patient.)

The fucose concentrations in the group of patients ($1,032.0 \pm 69.2$ µg/mL, median, 866.5; range, 210.0 to 3,039.0) were significantly higher than those in the controls (522.2 ± 80.5 µg/mL; median, 518.5; range, 150.0 to 983.0; $p = .0015$; Fig 2). The sialic acid concentrations in the group of patients (732.6 ± 33.9 µg/mL; median, 714.5; range, 178.0 to 1,498.0) were significantly higher than those in the controls (343.3 ± 32.9 µg/mL; median, 329.0; range, 209.0 to 569.0; $p < .0001$; Fig 2). In the 4 patients who complained of a globus sensation in spite of medication, the fucose and sialic acid concentrations of the mucus collected 2 weeks after the first sampling were also higher than those in the controls (Fig 2).

The TTS correlated significantly with the fucose concentrations ($p = .0316$; Fig 3). There was no significant correlation between the TTS and the sialic acid concentrations ($p = .6993$; Fig 3).

DISCUSSION

In all of the patients with globus pharyngis, secretions could be collected in the epipharynx. On the other hand, in healthy subjects, nasal and epipharyngeal secretion is so slight that sampling of measurable amounts of secretions for biochemical analysis is difficult; thus, nasal provocation tests have been performed.⁸ Different types of provoking agents, including methacholine, histamine, and allergen, have been used.⁸ Brofeldt et al⁸ reported that methacholine-induced secretions contained less fucose (295 µg/mL) than did histamine-induced (383 µg/mL) and allergen-induced secretions (422 µg/mL), while there were no significant differences in the sialic acid content of methacholine-, histamine-, and allergen-induced secretions (231, 233, 208 µg/mL, respectively). The concentrations of fucose and sialic acid in the acetylcholine-induced secretions in our study

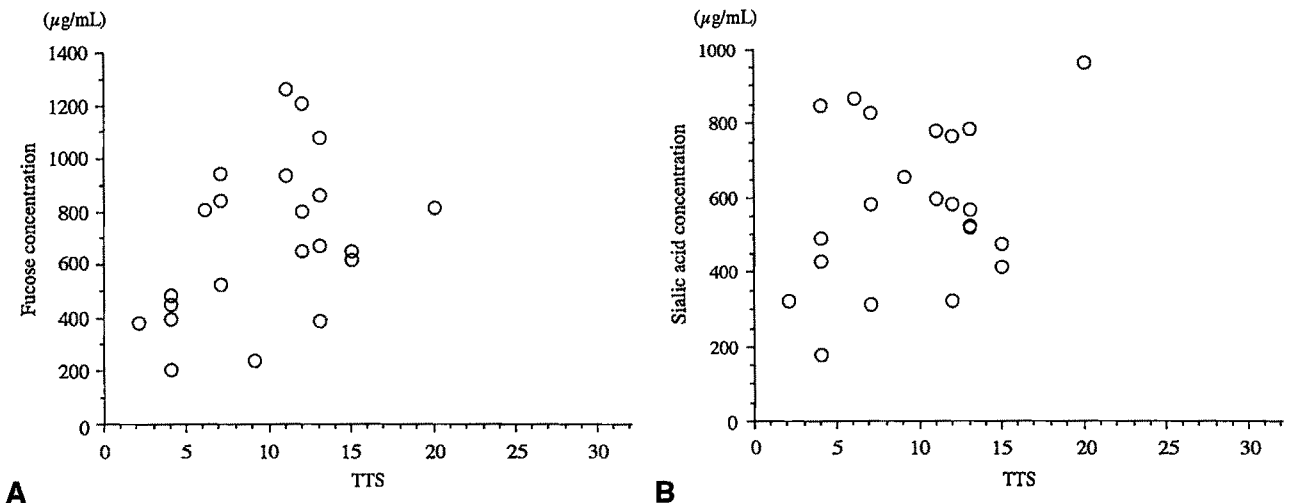


Fig 3. Spearman rank correlation analysis of **A)** fucose and **B)** sialic acid concentrations against total throat score (TTS).

were higher than those in the methacholine-induced secretions mentioned above. The epipharyngeal mucus of patients with globus pharyngis contains more fucose and sialic acid than do secretions induced by any provoking agent. As only 3 out of 40 patients had a nasal smear positive for eosinophils in this study, acetylcholine, which directly stimulates glandular cholinceptors,^{9,10} was thought to be most appropriate for the provocation test in healthy volunteers.

Fucose and sialic acid are markers of mucous glycoproteins. The concentration of fucose is proportional to the viscoelasticity of the nasal mucus.⁴ Fucose is present in respiratory mucous glycoproteins and absent from serum glycoproteins.⁹ Accordingly, fucose is the most important determinant of the rheological properties of nasal mucus. On the other hand, sialic acid is present in similar amounts in mucous and serum glycoproteins.¹¹ Sialic acid has an axial carboxylic group and, because of its negative charge, is considered to play a central role in establishing cross-links between glycoproteins.¹² Therefore, sialic acid has also been considered an index of mucus viscosity. The significantly higher concentrations of fucose and sialic acid found in patients with globus pharyngis support our hypothesis that sticky mucus anchored to the epipharynx may cause the globus sensation.

Every time we checked the epipharynx of patients with a persistent globus sensation by nasal endoscopy, there was a colorless, translucent mucus standing in the epipharynx. The concentrations of fucose and sialic acid were also higher than those in controls 2 weeks after the initial sampling in the 4 patients who complained of a persistent globus sensation.

tion in spite of 2 weeks of medication. In them, the sialic acid concentrations slightly changed for 2 weeks, while the fucose concentrations tended to be stable. This finding coincided with the significant correlation observed between the fucose concentrations and the TTS, which is associated with the severity of globus symptoms.

Secretions collected in the nasopharyngeal space are produced by goblet cells and seromucous glands in the upper part of the nasopharynx and the nose.¹³ The secretions from the posterior part of the nose show a significantly higher spinability than do those from the anterior part.¹⁴ In the paranasal sinuses, the number of glands is very limited,¹³ and the mucus production from the glands in the paranasal sinuses can be considered almost negligible.¹² In none of the patients did we observe apparent discharge from the orifice of the paranasal sinuses. The fact that in contrast to healthy subjects, some amount of mucus could be sampled without provocation from all patients may indicate an increased production of mucus in the posterior part of the nose and the epipharynx.

Hyperviscoelastic epipharyngeal mucus disturbs mucociliary transport.⁴ Some amount of mucus of high viscosity and spinability stagnant in the epipharynx in spite of swallowing and mucociliary transport, which may be sensed as a foreign body on swallowing, would cause the globus sensation.

The precise mechanism and production site of epipharyngeal mucus of high viscosity is to be elucidated in the future. However, we conclude that there is a close association between stagnant mucus in the epipharynx and the globus sensation.

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RAT LARYNGEAL TRANSPLANT MODEL: TECHNICAL ADVANCEMENTS AND A REDEFINED REJECTION GRADING SYSTEM

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The rat laryngeal transplant model, introduced in 1992, laid the basic science foundation that contributed to the first successful human larynx transplant in 1998. Over 1,500 rat transplants later, numerous modifications have improved the model, increasing the initially reported evaluability rate of 50% to almost 100%. The observed histologic rejection process has been altered, as well. We report the technique modifications, as well as the results of a new study using nonimmunosuppressed, allogenic transplantations, in order to define a new rejection grading system. Using the updated model, we performed 50 transplantations between LBN(f1) donor rats and Lewis recipients. Larynges from 8 groups of 5 to 10 animals were harvested at intervals between 1 and 20 days after transplantation. The larynges were examined grossly and microscopically in a blinded manner for evidence of rejection. A multivariable linear regression model was used to define a new rejection grading scale. All 50 animals survived their assigned posttransplantation period. No animals exhibited vascular thromboses, for an evaluability rate of 100%. Histologic criteria in 7 categories and gross criteria in 5 categories demonstrated increased rejection proportional to the amount of time after transplantation. The equation $[\text{Group} = -2.209 + 0.465 \times (\text{Size}) + 0.901 \times (\text{VA Flow}) + 0.613 \times (\text{Muscle}) + 1.040 \times (\text{Thyroid})]$ reproducibly grades rejection on the basis of gross and histologic findings. New modifications to the rat laryngeal transplant model have conferred greatly improved animal survival rates and anastomotic patency rates. Additionally, the observed rates of rejection have been reduced in comparison to those of initial studies. This updated rejection staging system will be used to compare immunosuppressive regimens in future rat laryngeal transplant studies.

KEY WORDS — larynx, rat, rejection, thyroid cartilage, transplantation.

INTRODUCTION

The first animal model for laryngeal transplantation was the canine model, described in 1965.¹ After Work and Boles determined that the dog larynx could survive pedicled on the superior laryngeal artery and vein alone, they performed a successful reimplantation of an autologous larynx including vessel anastomoses.² Soon after, Ogura et al³ described the first successful homologous transplantation using the canine model.

The first successful laryngeal transplant in a human was performed on January 4, 1998, by the senior author (M.S.). The transplanted complex included the larynx, pharynx, 5 rings of trachea, the thyroid gland, and the parathyroid glands. The patient continues to function well to date with his transplanted organs.⁴ The rat model for laryngeal transplantation was introduced by the senior author in 1992, which subsequently laid the groundwork of basic science knowledge that made the attempt at human transplantation successful.⁵ The initial de-

scription of this model reported that the laryngotracheal complex was placed in tandem with the native airway and used an arteriovenous shunt for the venous outflow through the superior thyroid artery. Of the 16 rat transplantations performed, death occurred in 13%; the remaining rats demonstrated a 64% arterial patency rate and a 43% venous patency rate. The benefits of the rat model were reported as low cost, a dose-related sensitivity to medications that more closely resembled that of humans than did that of dogs, and the ability to perform trials that included large numbers of animals.

Subsequent work by the same group defined the histopathologic correlates one would expect to see in rats after transplantation without immunosuppression.⁶ Although there were no deaths associated with the 24 transplantations, 50% of the organs demonstrated infarction upon microscopic evaluation. Of the transplants that survived, changes to specific tissues (mucosa, minor salivary glands, cartilage, and blood vessels) were graded and used to formulate a

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grading scale for laryngeal rejection.

Over the past 10 years, numerous modifications have been made to the initial technique described in 1992. Along with improved rates of survival and evaluability, the rate of microscopic rejection has been altered. It is the purpose of this article to report the modifications made to the originally described model and to describe in detail the currently used technique. A new study involving 50 allograft transplantations without immunosuppression was performed with planned sacrifice between 1 and 20 days after transplantation. Structures were then graded in a blinded manner for specific changes. A multivariable linear regression model was then used to establish a new grading system to be used in subsequent trials that will evaluate various immunosuppressive regimens.

MATERIALS AND METHODS

This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The Cleveland Clinic Foundation.

CURRENT RAT LARYNGEAL TRANSPLANTATION TECHNIQUE

Donor Harvest. Lewis Brown Norway male rats, first generation (LBNf1), between the ages of 6 and 8 weeks serve as donors (Harlan Laboratories, Indianapolis, Indiana). Their weight at the time of harvest is between 175 and 200 g. Chloral hydrate, 12 mg/100 g body weight, is injected intraperitoneally for sedation. The skin over the neck is swabbed with Betadine and resected. The salivary glands are removed, the strap muscles are separated in the midline, and a tracheostomy is performed between the fourth and fifth tracheal rings. Both vagal nerves are resected, and the internal and external carotid arteries are isolated. The bilateral internal carotid arteries are ligated, as well as the external carotid arteries superior to the superior thyroid arteries, such that the laryngotracheal complex is pedicled solely upon the superior thyroid arteries. Heparin sodium is injected in the external jugular vein at 2 U/g. Bovie cautery transects the tissues between the larynx and the hyoid bone and is continued posteriorly through the pharynx. Temporary clamps are placed on both common carotid arteries, which are transected proximally. The trachea is divided at the tracheostomy site, and the esophagus is transected posteriorly at the same axial level. Pentobarbital sodium 50 mg is injected intravenously for euthanasia.

Preparation of Graft. Within a sterile solution of

heparinized saline, the carotid arteries are manually milked free of blood. Heparinized Ringer's solution (200 U/10 mL) is gently injected through a 22-gauge needle to flush the organ until there is no visibly bloody discharge from the opposite carotid artery. Then, 6 mL of Wisconsin's solution (ViaSpan, Belzer UW, DuPont Pharmaceuticals, Wilmington, Delaware) is injected and the organ is placed into cold Wisconsin's solution during preparation of the recipient.

Transplantation. A male Lewis rat, 6 to 8 weeks old, is sedated in the same manner as in the organ harvest. The neck is prepared with Betadine, and a 3-cm vertical incision is performed. The left salivary gland is resected, as well as the left strap muscles. The left carotid artery is isolated and temporarily clamped. The donor organ is then flushed with heparinized saline solution and placed into the field. The donor's common carotid arteries are shortened to 5 mm in length with a beveled cut. An arteriotomy is performed in the recipient's left common carotid artery, and an end-to-side anastomosis is performed by using 10-0 monofilament nylon in a continuous fashion under the operative microscope.

The left external jugular vein is isolated and temporarily clamped. An end-to-side anastomosis is performed in a similar manner to the first. One hundred units of intravenous heparin sodium is injected systemically, and all clamps are released. Reperfusion of the organ is easily confirmed by an immediate "flush" of the thyroid gland and visible blood flow through the anastomoses. The field is flushed with penicillin (30,000 Ambi-Pen, Anthony Products, Irwindale, California), and the incision is closed with 4-0 silk.

Postoperative Care. Immediate injection of 30,000 U of penicillin is performed subcutaneously. The rats are housed in microisolator cages with specially designed filter lids to prevent exposure to ambient bacteria or viruses. The cages are fitted with mesh bottoms that keep soilage away from the surgical incision. The animals drink water with 1 mg/mL tetracycline hydrochloride (Barr Laboratories, Pomona, New York) and 1 mg/mL acetaminophen throughout their postoperative course at a rate of approximately 30 mL/d. Acetaminophen 16 mg per dose is given via gavage immediately after the operation and then as needed.

Sacrifice and Histologic Evaluation. Sedation is repeated as outlined above. The incision is opened, and both vascular pedicles are inspected for thromboses. The amount of fibrous capsulization is noted, and the larynx is entered superiorly to document the production of secretions intraluminally. Both pedicles

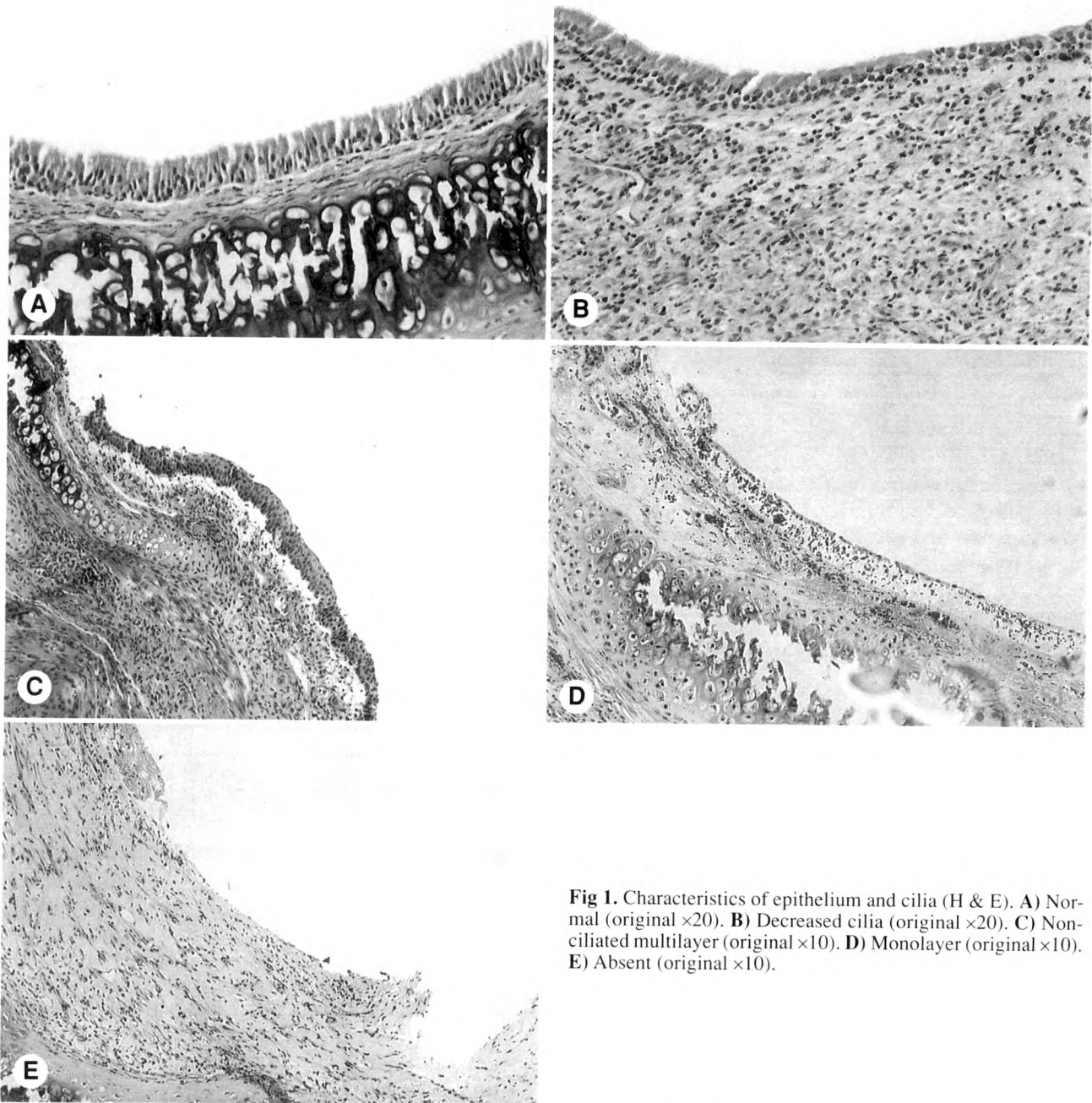


Fig 1. Characteristics of epithelium and cilia (H & E). **A)** Normal (original $\times 20$). **B)** Decreased cilia (original $\times 20$). **C)** Non-ciliated multilayer (original $\times 10$). **D)** Monolayer (original $\times 10$). **E)** Absent (original $\times 10$).

are transected, and the organ is placed into 10% formaldehyde without flushing. Any blood needed for drug levels or lymphocyte analysis is then drawn, and the animal is painlessly sacrificed with pentobarbital sodium 50 mg intravenously. The larynges are embedded in paraffin and sectioned at 4- μ m thickness. After staining with hematoxylin and eosin, axial sections are plated for histologic review.

Trial Design for New Rejection Grading System. By the above technique, 50 transplantations were performed between LBN(f1) and Lewis rats. The subjects were given no immunosuppression after the op-

eration. Sacrifice was performed at 1 day, 3 days, 5 days, 7 days, 9 days, and 12 days (5 rats each) and at 15 days and 20 days (10 rats each).

All organs were graded at the time of harvest for 5 gross findings: encapsulation by fibrous tissue (absent, mild, severe), size (normal, atrophic, enlarged), arterial flow (normal or occluded), venous flow (normal, decreased, or occluded), and amount of intraluminal secretions (absent, mild, or profuse). All slides were evaluated histologically by the same reviewer in a blinded fashion. Seven structures were graded: epithelium (5 grades), muscle, minor salivary glands,

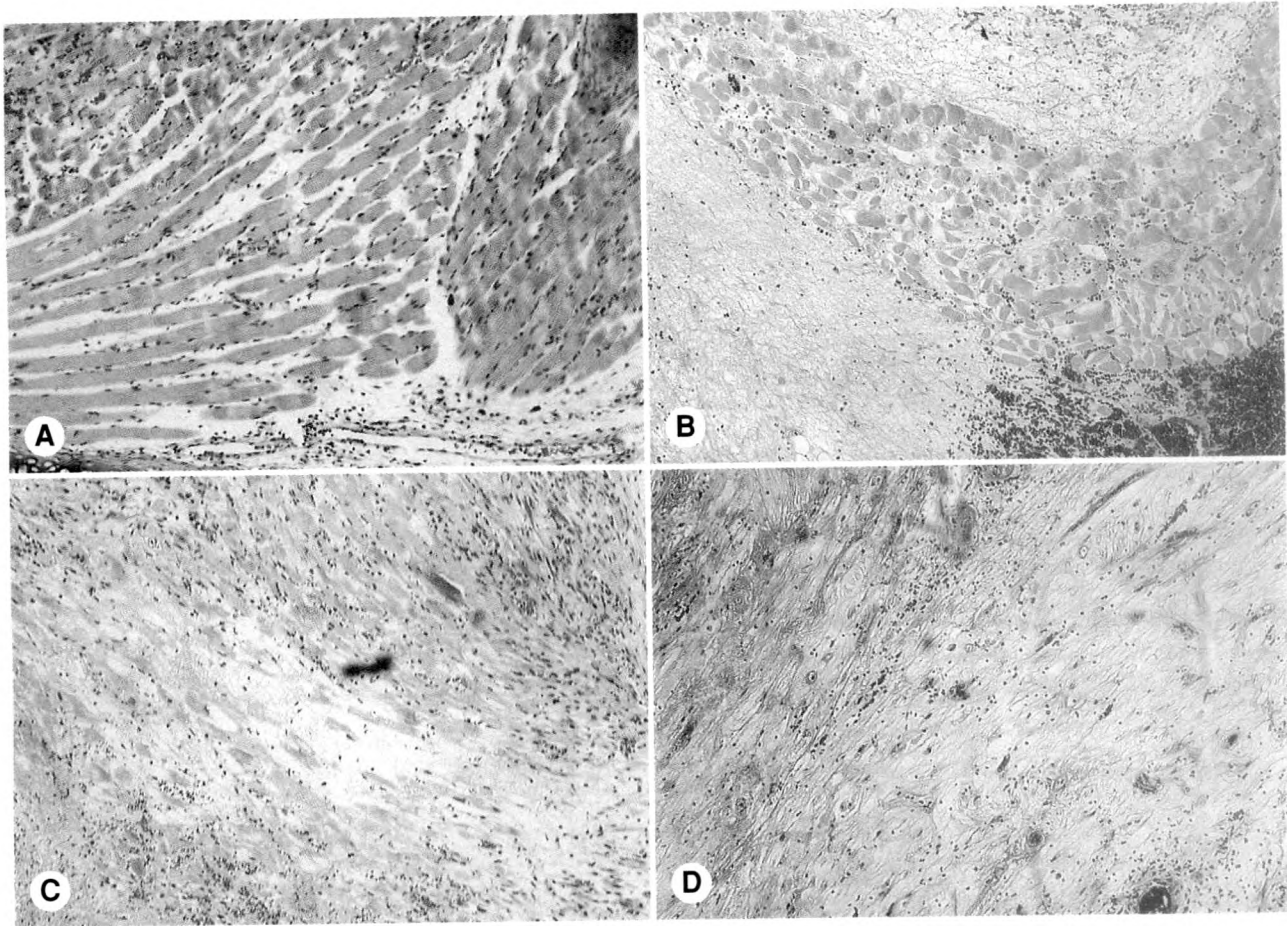


Fig 2. Characteristics of muscle (H & E, original $\times 10$). **A)** Normal. **B)** $>50\%$ Nucleated. **C)** $<50\%$ Nucleated. **D)** Absent.

lymphocytic infiltration, and thyroid gland (4 grades), perichondrium (3 grades), and cartilage (2 grades). Examples of grading are given in Figs 1-3.

STATISTICAL ANALYSIS

Model Building. After each organ had been graded in the 5 gross and 7 microscopic categories, a linear regression model was constructed to determine which characteristics were the most meaningful in predicting from which time period the specimens had originated (Statistical Analysis System, version 6.12, SAS Institute, Cary, North Carolina). A backward stepwise selection with an exit criterion of $\alpha = 0.05$ was used to remove variables that seemed to contribute little toward explaining the variability of the groups.

Model Assessment. The derived equation was then used to test the model's accuracy. This was done by re-deriving the equation with the results of 49 of the specimens and testing the one organ taken out of the analysis. In this way, every one of the organs was tested to determine how similar the predicted group would be without its own bias in the equation. These actual groups versus these predicted groups were then charted.

Collapsing of Groups. Eight separate categories would be unwieldy for assessing future organs for signs of rejection. Therefore, adjacent groups were combined in a manner that would preserve the maximum predictability of the category. Last, each individual specimen was reanalyzed, and the specific gross and histologic characteristics of each time grouping were determined.

RESULTS

All 50 animals survived the transplant procedure and their preassigned posttransplantation period, for 100% survivability. There were no signs of infection or outward signs of rejection, even in the 20-day period.

All specimens were successfully graded in the 5 gross categories and the 7 microscopic categories for a total of 600 data points. Of note, all 30 specimens between 1 day and 12 days had normal flow in both the anastomosed artery and vein. All 10 specimens at 20 days exhibited decreased venous flow through the anastomosed vein but normal arterial patency and no evidence of thrombosis. Other general findings were absent secretions for all 50 specimens and normal size for the 40 specimens 15 days or less after

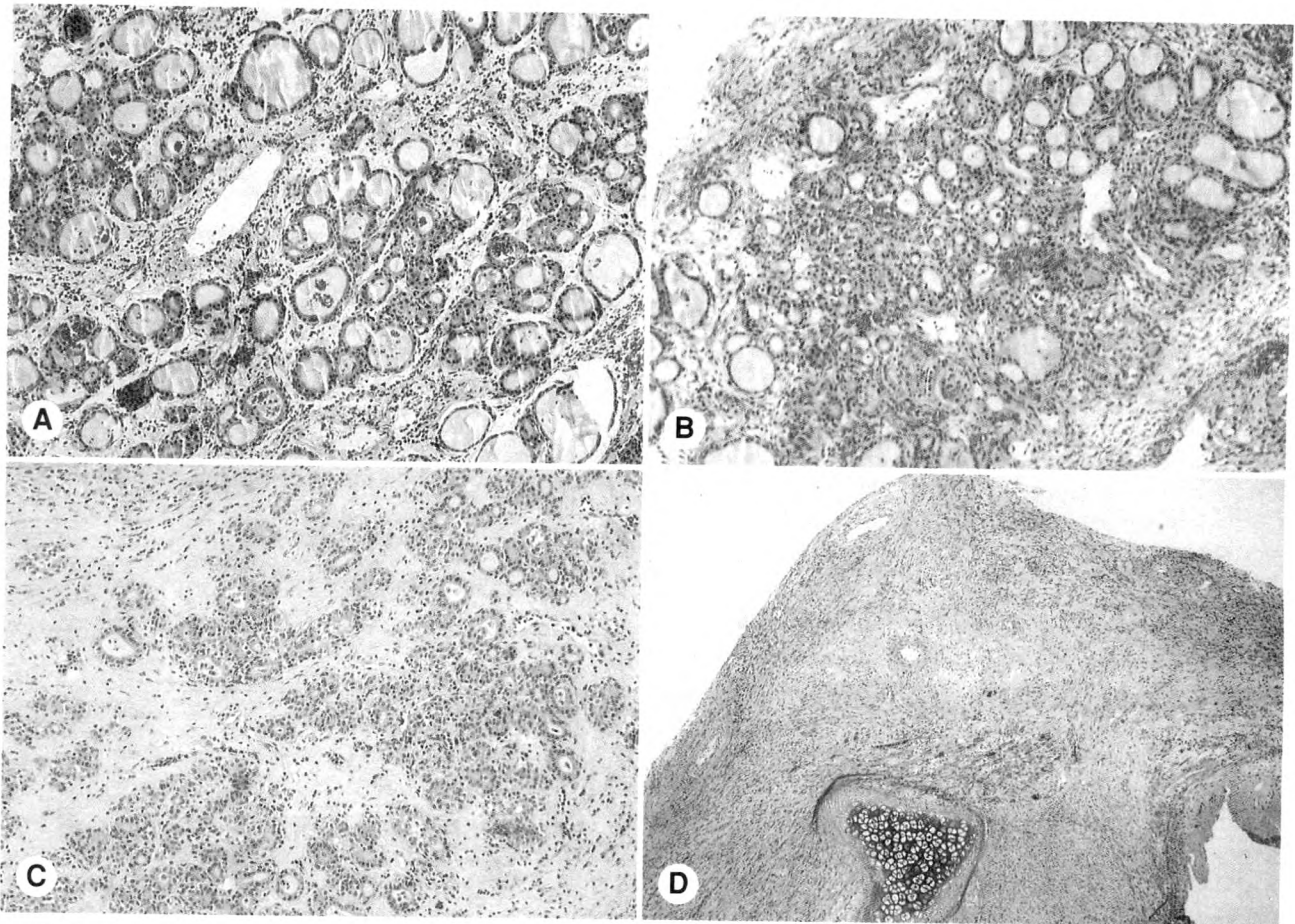


Fig 3. Characteristics of thyroid gland (H & E). **A)** Normal (original $\times 10$). **B)** $>50\%$ Density (original $\times 10$). **C)** $<50\%$ Density (original $\times 10$). **D)** Absent (original $\times 4$).

transplantation, whereas all 10 specimens at 20 days appeared atrophic in size. Microscopic findings demonstrated progressive destruction of all of the structures that were graded, with the exception of lymphocytic infiltration. In this category, minor infiltration was present at 1 day after transplantation, which then peaked to profound infiltration at 5 days after transplantation (all 5 animals in the group). Subsequent infiltration then decreased to moderate and again to mild with progressively later time intervals.

After constructing a multivariable linear regression model to be able to predict rejection using the results from these 50 rats, we determined that the most useful categories for predicting rejection were size of the specimen, venous flow, and muscle and thyroid destruction. Graded severity in these categories was then assigned numeric values, eg, for muscle, grade 1 means normal, grade 2 means $>50\%$ nucleated, grade 3 means $<50\%$ nucleated, and grade 4 means absent. Table 1 demonstrates how the following multivariable linear regression equation would predict the placement of each specimen into a specific grouping compared to their actual group: $\text{Group} = -2.209 + 0.465 \times (\text{Size}) + 0.901 \times (\text{VA Flow}) +$

$$0.613 \times (\text{Muscle}) + 1.040 \times (\text{Thyroid}).$$

A correct classification based on the linear regression modeling procedure would be made for 27 (54%) of the 50 subjects. Classifying rejection into 8 different severities is not only cumbersome, but is not very useful for comparing subsequent immunosuppressive regimens. Therefore, the outlined boxes in Table 1 demonstrate the "collapsing" of groups from 8 separate ones into 5: (1), (2), (3,4), (5,6), and (7,8). Although less specific in their definitions, the rate of correct classification based on the linear regression model improves to 40 of 50 subjects (80%) with this system. Only 1 subject (in italics) is predicted incorrectly from the actual time period by more than 1 group (2%).

These groups were subsequently named according to their time after transplantation: normal (24 hours), minimal rejection (3 days), early moderate rejection (5 to 7 days), late moderate rejection (9 to 12 days), and severe rejection (15 to 20 days). The original characteristics of each specimen were then correlated with the amount of time after transplantation and are charted as a descriptive classification scheme in Table 2.

TABLE 1. TESTING ACCURACY OF MULTIVARIABLE LINEAR REGRESSION MODEL IN PREDICTING REJECTION GRADE

Actual Group	Predicted Group							
	1	2	3	4	5	6	7	8
1	4		1					
2		5						
3			3	2				
4			1	1	2	1		
5				2	2	1		
6					1	4		
7					1	2	1	6
8						1	2	7

DISCUSSION

To summarize our findings, our rat laryngeal transplant model as described was able to achieve 100% survivability and 100% evaluability with no infarctions secondary to thrombosis out of the 50 transplantations performed. In addition, a new histologic grading system is described based upon the chronological rejection observed at the ultrastructural level with no immunosuppression given. Using the histologic criteria, one is able to accurately predict the amount of rejection time allowed to occur with 80% accuracy among the 5 different time periods, and to

98% accuracy within 1 adjacent time period.

The model described is the evolution of Strome's rat laryngeal transplant model, initially reported in 1992.⁵ Unfortunately, the original technique conferred a 13% perioperative mortality rate, as well as a 36% arterial thrombosis rate and a 57% venous thrombosis rate for the 14 surviving animals. Since that initial report, 6 major changes in the model have been instituted.

The use of Wisconsin's solution was investigated in 1994 and was shown to significantly prolong the maximum tolerated ischemia time from 3 to 20 hours, so it is now used in all transplantations.⁷ As originally described, cautery was used after reperfusion to control bleeding. Cautery is now avoided to minimize tissue injury, and the majority of bleeding sites have been found to be self-limiting. Although no frank purulence was evident in earlier studies, several changes have been made to decrease possible bacterial contamination. Penicillin solution is now used to bathe the operative field at the conclusion of the transplantation. New mesh-bottom cages keep excrement away from the ventrally placed neck incision and are fitted with filter lids to decrease ambient bacterial and viral exposure. Last, the technical

TABLE 2. GROSS AND HISTOLOGIC CHARACTERISTICS OF REJECTION OBSERVED AFTER TRANSPLANTATION

	Normal (24 h)	Minimal (3 d)	Early Moderate (5-7 d)	Late Moderate (9-12 d)	Severe (15-20 d)
Gross findings					
Encapsulation (absent, mild, severe)	Absent	Mild	Mild	Severe	Severe
Size (normal, atrophic, enlarged)	Normal	Normal	Normal	Normal	Normal at 15 days; atrophic at 20 days
Arterial anastomosis flow (normal, occluded)	Normal	Normal	Normal	Normal	Normal
Venous anastomosis flow (normal, decreased, occluded)	Normal	Normal	Normal	Normal	Decreased
Secretions (absent, mild, profuse)	Absent	Absent	Absent	Absent	Absent
Histologic findings					
Epithelium and cilia (normal, decreased cilia, multilayer, monolayer, absent)	Decreased cilia	Multilayer	Multilayer	Monolayer	Absent
Muscle (normal, >50% nucleated, <50% nucleated, absent)	Normal	Normal	>50% Nucleated	Absent	Absent
Minor salivary glands (normal, dilated, atrophied, absent)	Dilated	Dilated	Atrophied	Absent	Absent
Lymphocytic infiltration (absent, minor, moderate, profound)	Minor	Moderate	Profound at 5 days; minor at 7 days	Minor	Minor
Thyroid gland (normal, >50% density, <50% density, absent)	Normal	>50% Density	<50% Density	Absent	Absent
Perichondrium (normal, >50% nucleated, <50% nucleated)	Normal	Normal	Normal	>50% Cellularity	<50% Cellularity
Cartilage (normal, decreased cellularity)	Normal	Normal	Normal	Normal	Decreased cellularity

aspects of the microvascular anastomoses have been changed. Continuous suture technique is now performed instead of interrupted suturing, and the venous anastomosis is designed to be in a more perpendicular end-to-side relationship with the external jugular vein.

Not only did the survivability and evaluability of the model improve after these changes were incorporated, but the rate of rejection has slowed in these nonimmunosuppressed control experiments. In the initial 1992 report of histopathologic findings, laryngeal muscle was lost by postoperative day 5; it is currently observed to persist until day 9. One week after transplantation, initial reports demonstrated destruction down to a "monolayer" epithelium; this is currently not observed until day 9. One used to expect overall loss of ultrastructural architecture and replacement by granulation tissue by 14 days after transplantation, whereas we now see decreased cellularity of tissues and preservation of overall architecture even at 20 days after transplantation. The grading of thyroid gland replacement by inflammatory cells and a more defined grading of muscle loss have been added to the rejection grading system, as well.

Findings similar to our own have been seen in laryngeal transplants in the canine model. Silver et al⁸ performed 11 allograft transplantations in dogs without immunosuppression to determine the nature and timing of rejection. In the 6 animals that survived 8 days or more, mucosal necrosis occurred by day 12. Serial biopsies demonstrated an early submucosal infiltrate that tended to regress by the fifth postoperative day, similar to our own peak of "profound" lymphocytic infiltration. This time period likely represents the height of antigenicity of the transplanted organ. Unlike in the rat model, the dogs' transplanted tissue exhibited almost complete necrosis by the 10th day except for cartilage, which remained intact even at 2 weeks after operation.

The rat has been used as an animal model to study other organ transplantation techniques. The sequence of histologic rejection in the rat cardiac model was

studied by Tilney et al.⁹ Using LBN(f1)-to-Lewis strain transplantations, the authors noted an intense cellular infiltrate in the subendocardium by 3 days after transplantation. Between 4 and 5 days, interstitial hemorrhage had occurred, with lymphocytes collecting between muscle fibers, and myocardial necrosis was widespread. By 7 days, there was cessation of ventricular contractions, with little histologic myocardium remaining. Prop et al,¹⁰ studying rat combination heart-lung transplants, found that lung tissue deteriorates at a faster rate than heart tissue. In LBN(f1)-to-Lewis strain transplants, alveoli had undergone functional rejection by 6 days, whereas cardiac grafts appeared to remain viable for as long as 11 days.

The rat model remains a powerful tool for studying rejection of laryngeal transplantation. A skilled microsurgeon can perform a complete harvest and transplantation in less than 2.5 hours. Combined with low cost and relatively little postoperative care as compared to other animals, the rat model has allowed our laboratory to perform more than 1,500 transplantations to study multiple immunosuppressive regimens, including medications such as cyclosporine, FK-506, rapamycin, mycophenolate mofetil, and methylprednisolone.¹¹⁻¹³ Whole-body irradiation, donor-specific transfusion, and irradiation of the donor organ in vitro have been studied, as well.¹⁴ It is our goal to transfer the knowledge gained from this animal model to humans, with the ultimate goal of human laryngeal transplantation immunosuppression with no co-morbidities.

CONCLUSION

Laryngeal transplantation may be performed in the rat model with excellent survivability and evaluability. Technical modifications to the original model reported in 1992 have altered the progression of rejection observed when no immunosuppression is given. By use of the described chronological sequence of rejection as a grading system, subsequent studies will evaluate the immunosuppressive effects of various medical regimens in this rat model.

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COMPUTER AIDED SURGERY AROUND THE HEAD (CAS-H)

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EFFECTS OF CLARION ELECTRODE DESIGN ON MAPPING LEVELS IN CHILDREN

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The design of the placement of the Clarion cochlear implant's intracochlear electrode array has undergone 2 revisions since its introduction, each to improve modiolar proximity. Stimulation with modiolar proximity may reduce current requirements for threshold levels and most comfortable levels of stimulation. This study analyzed the effects of electrode design on programming levels for deaf children implanted with the 3 cochlear implant designs and followed at The Children's Hospital of Philadelphia. Psychophysical data were reported if measurements were taken approximately 3 months after initial activation, and programming parameters included nonsequential monopolar stimulation of 75- μ s-per-phase biphasic pulses presented at 813 Hz per electrode. The threshold level and most comfortable programming level were measured by standard clinical techniques appropriate for children. The results indicate that the 2 electrode placement revisions have each significantly reduced threshold levels and most comfortable stimulation levels. These results are discussed in the context of device aesthetics, safety, and function.

KEY WORDS — child, cochlear implant, electrode impedance, modiolar proximity.

INTRODUCTION

The design of the Clarion intracochlear electrode array has undergone 2 major revisions since being introduced in March of 1996. Each Clarion intracochlear electrode array has been designed so that it may be positioned close to the modiolus. It may be anatomically advantageous to position the electrode array in this manner, because 1) the modiolus contains the neural elements with which the cochlear implant interfaces, 2) closer placement of the electrode array against the modiolar wall may prohibit the growth of fibrous tissue between the array and the neurons, and 3) reduced channel interactions may be seen.^{1,2} These theories are supported by Shepherd et al,³ who found an increase in the electrically evoked auditory brain stem response thresholds with increased distance from the modiolus.

The first commercially available version of the Clarion electrode array consisted of a 25-mm silicone rubber carrier surrounding a polyester sleeve, in which 16 Teflon-coated electrode wires were housed. The active electrode array length was 14.5 mm.⁴ The silicone carrier varied in dimensions to conform to the shape of, and was precurved to maintain its position close to, the modiolar wall of an average cochlea. It was designed to reach one and a half turns (540°) into the cochlea. The surface area of each ball electrode was 0.012 mm². The electrode design underwent a minor modification of electrode

wiring and was then marketed as the "enhanced bipolar" electrode array. The first major revision to the design of the placement of the array was the electrode positioner system (EPS), introduced in 1998. This design did not change the intracochlear electrode array of the implant. Rather, the EPS was a separate, precurved piece of silicone rubber, thinner at the distal end. The EPS was concave on the medial side, to fit the intracochlear array, and convex on the lateral side, to come into contact with the lateral wall of the cochlea. The EPS was shorter than the electrode array, designed to make one complete turn (360°) into the scala tympani.⁵ The second major change involved the design of a new intracochlear electrode array called the HiFocus. The HiFocus array consisted of a silicone rubber carrier precurved, not to the shape of the cochlea, but rather, to conform to the curvature of the basal turn and to orient the electrode contacts to the medial side of the cochlea. The array was designed to be inserted one and three quarters turns (630°), with an active electrode length of 17 mm. The surface area of each plate electrode was 0.20 mm². The HiFocus was designed to be used with a separate positioner.

To date, there have been no published reports as to the effects of the various Clarion electrode designs on psychophysical threshold and maximum comfortable levels of stimulation in human subjects. Unpublished conference proceedings and US Food

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and Drug Administration clinical trial reports have described reduced programming levels with the spiral array with the EPS, compared to the spiral array without the EPS, and further reduced levels with the HiFocus array with the positioner (unpublished observations by M. Osberger et al, 1999 and 2000; T. Lenarz et al, 1999; R. D. Battmer et al, 2000; and Advanced Bionics Corporation, Sylmar, California, 1999).

The purpose of this study was to examine the effects of Clarion electrode design on threshold and most comfortable loudness levels in children. It was hypothesized that lower stimulation levels would result with the use of the EPS with the enhanced bipolar electrode design because of the closer placement of the array with respect to the modiolus. Furthermore, it was hypothesized that lower stimulation levels would result from the HiFocus electrode design because of the orientation of the electrode contacts toward the modiolus.

METHODS

Data for this study were taken from pediatric deaf subjects who received cochlear implants and were followed at The Children's Hospital of Philadelphia (Philadelphia, Pennsylvania). All of the subjects in this study used cochlear implant systems manufactured by Advanced Bionics Corporation. The subjects used the enhanced bipolar electrode design, the enhanced bipolar electrode design with the EPS, or the HiFocus electrode design with the EPS.

Data were included in the analysis if programming parameters included nonsequential monopolar stimulation of 75- μ s-per-phase biphasic pulses presented at a rate of 813 Hz per electrode, which is the default programming parameter set for stimulation with the continuous interleaved sampling (CIS) programming strategy with the Clarion cochlear implant system. The mapping levels closest to 3 months after activation were included in the analysis. Children for whom mapping level data were only available less than 1 month or more than 6 months after activation were excluded.

The details of the cochlear implant system used in this study are described above in the Introduction. The design of the internal controller stimulator (ICS 1.2) was identical for the 3 electrode designs.

Programming levels were established with SCLIN for Windows version 98.004 (Advanced Bionics Corporation, 1998) and SCLIN2000 version 1.03.06.001 (Advanced Bionics Corporation, 2000). This software defines stimulation parameters that are passed to a speech processor by a clinical programming in-

terface. The speech processor then sends stimulation parameters to the ICS by radio frequency pulses. The transmitting and receiving antennae are aligned and held in place by external and internal magnets.

Threshold programming levels (T-levels) were determined by means of behavioral observation audiometry, conditioned play audiometry (CPA), or voluntary response techniques. In CPA, the child is taught to respond to stimulation by performing a simple task. For example, a child might drop a block in a bucket or place a peg in a pegboard in response to stimulation. Ideally, the T-level is established when the child first responds twice to ascending stimulus levels. The clinician is able to monitor false-positive rates by varying the interval between stimuli. The voluntary response technique involves the child in reporting the presence of stimulation by raising his or her hand or nodding when a stimulus is perceived. As with CPA, false-positive responses are monitored by varying the presentation interval. When a high false-positive rate is detected by the clinician, the task is altered to reorient the child to the task.

Most comfortable loudness levels (M-levels) are determined by raising the level of stimulation until the child expresses that the stimulation is at the upper limit of comfort. If the child expresses that the stimulus is uncomfortable, the stimulus level is decreased. A child might express that a sound is uncomfortably loud by telling the clinician to stop or by more subtle cues such as reddening in the face or an upturned bottom lip. After the levels have been assigned across the electrode array, modifications may be made in a live-voice mode to globally increase or decrease the M-levels, depending upon the reaction of the child.

In the clinical fitting environment, the "clinical unit" (CU) is the unit used to measure the stimulation level. The CU levels are equal to the commanded current measured in microamperes. There is a nonlinear relationship between commanded current and actual current output by the device that is highly dependent on the impedance of the stimulating electrode. This relationship is roughly 1:1 for low commanded currents, but becomes compressive at higher commanded current levels (unpublished observations by Advanced Bionics Corporation, 1999).

In monopolar stimulation modes, T-levels and M-levels can be interpolated between tested electrodes. By the time of the programming session for which levels were used in this experiment, all electrodes had been individually tested.

The electrode impedances were measured with the SCLIN for Windows and SCLIN 2000 software pack-

TABLE 1. SUBJECT DETAILS

Implant Design	No. of Subjects	Meningitis as Cause		Congenital Deafness		Age* (y)		Days After Activation*	
		No.	%	No.	%	Average	Range	Average	Range
Enhanced bipolar	31	5	16	20	65	4.4	1.8-14.2	95	42-173
Enhanced bipolar with EPS	12	1	8	8	67	6.8	2.0-12.1	97	63-157
HiFocus with EPS	18	3	17	12	67	5.7	1.7-15.6	83	31-168

EPS — electrode positioner system.
*At time programming levels were measured.

ages. The software used a single-point measurement of a 1,000-Hz sine wave to measure the impedance values.

The average electrode stimulation levels were compared using 2-tailed *t*-tests assuming unequal variances.

RESULTS

The stimulation levels for 61 subjects were compared for the 3 electrode designs. Subject characteristics, grouped by cochlear implant design, are included in Table 1.

At the time of the visit when mapping levels were established, all of the subjects using the enhanced bipolar array and the enhanced bipolar array with EPS were programmed with the CIS speech processing strategy. Of the subjects using the HiFocus array with EPS, 67% were programmed with the simultaneous analog stimulation (SAS) speech processing strategy. The remaining 33% of the HiFocus with EPS subjects were programmed with CIS.

With the enhanced bipolar array, the average T-levels and M-levels were significantly lower when the EPS was used ($p = .030$ and $p = .024$, respectively). With the EPS, the average T-levels and M-levels were lower when the HiFocus electrode array was used. This difference was statistically significant for the T-levels ($p = .049$; Fig 1 and Table 2).

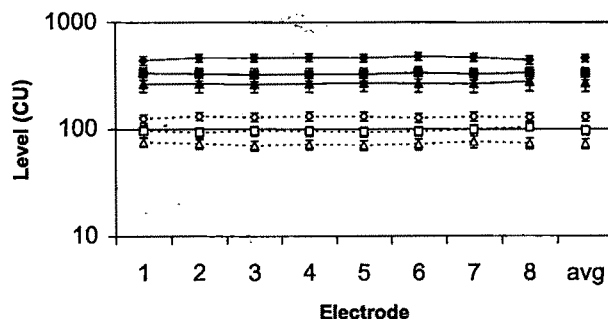


Fig 1. Threshold programming levels (T-levels; dashed lines and open symbols) and most comfortable loudness levels (M-levels; solid lines and filled symbols) for different electrode designs. Diamonds — enhanced bipolar array; squares — enhanced bipolar array with electrode positioner system (EPS); triangles — HiFocus array with EPS. Error bars indicate standard error of mean.

Only half of the 16 electrodes are used in CIS. Unused electrodes have relatively high impedances, and are excluded from this analysis. With the enhanced bipolar array, electrode impedance values did not significantly change with use of the EPS. Use of the HiFocus array resulted in lower impedance values than did use of the enhanced bipolar array with and without the EPS ($p = .041$ and $p = .054$, respectively; Fig 2 and Table 2). The difference in average electrode impedance values of the HiFocus and enhanced bipolar arrays does not result in enough of a difference in current output to account for the effects of electrode design on stimulation levels. The magnitude of the current output difference represents 28%, 23%, and 12% of the standard deviation of the M-levels of the average enhanced bipolar, enhanced bipolar with EPS, and HiFocus with EPS designs, respectively (unpublished observations by Advanced Bionics Corporation, 1999).

DISCUSSION

All electrode designs of the Clarion cochlear implant system were developed in order to provide stimulation in proximity to the modiolus. The design change from the enhanced bipolar array to the enhanced bipolar array with EPS sought to improve modiolar proximity by using static pressure generated from the lateral wall of the cochlea to hold the array closer than the precurved array alone. The HiFocus electrode array differed in the orientation of the stimulation sites in reference to the modiolar wall. The HiFocus array also took advantage of pressure

TABLE 2. PROGRAMMING LEVELS AND IMPEDANCE VALUES

Implant Design	T-level (CU)	M-level (CU)	Impedance (k Ω)
Enhanced bipolar	129 $\pm$ 62 (53-294)	459 $\pm$ 209 (86-933)	24 (17-67)
Enhanced bipolar with EPS	97 $\pm$ 30 (55-152)	328 $\pm$ 142 (141-654)	25 (19-42)
HiFocus with EPS	73 $\pm$ 32 (21-124)	267 $\pm$ 186 (68-730)	19 (9-42)

Data are average $\pm$ SD, with range in parentheses.

T-level — threshold programming level; M-level — most comfortable loudness level; CU — clinical units.

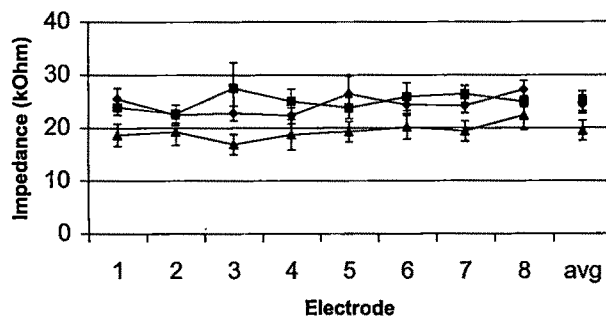


Fig 2. Impedance values for different electrode designs. Diamonds — enhanced bipolar array; squares — enhanced bipolar array with EPS; triangles — HiFocus array with EPS. Error bars indicate standard error of mean.

generated by the EPS.

The results from this study of 61 children using 3 electrode designs confirm that the design objective of reducing current requirements for stimulation has been accomplished in the clinical cochlear implant fitting environment. The required current levels were significantly lowered with the addition of the EPS to the enhanced bipolar array, and significantly lowered again by the HiFocus electrode design. These trends were similar to those in previous unpublished reports in adults, although in this study, stimulation levels across electrode designs were higher (unpublished observations by M. Osberger et al, 1999 and 2000; T. Lenarz et al, 1999; R. D. Battmer et al and Advanced Bionics Corporation, 1999). In another study reporting higher stimulation levels in children than in adults, this difference was presumed to be due to the fact that in children, maximum comfortable loudness is set below the level of tolerance, while in adults, it is set below the level of comfort. In addition, children's threshold levels may be set at minimal response levels, which may be suprathreshold.⁶

In the population studied, the preoperative diagnosis of meningitis did not preclude use of the EPS. A similar percentage of children with meningitis were implanted without (16%) and with (13%) the EPS. Therefore, possibly increased current level requirements in children with a history of meningitis would affect each electrode design population similarly. Likewise, the proportion of children whose deafness was congenital was similar across electrode types. Because of the similar ages of the children, the length of deafness was not a factor in this analysis.

The clinical techniques for determining psychophysical levels in children involve clinical interpretation and skill, and can be biased by the clinician and the subject. The clinicians who measured psychophysical levels in this study were experienced pediatric audiologists who specialized in caring for chil-

dren with cochlear implants. Until psychophysical testing paradigms can be implemented to reduce bias, such bias cannot be eliminated. The purpose of this study was not to compare stimulation levels across electrode designs by measuring them with rigorous psychophysical techniques. Rather, the purpose was to examine the levels as they are measured clinically.

Impedance measures did not change with the use of the EPS with the enhanced bipolar electrode array. This is an expected result, as the electrode design was identical and the same electrodes were used in the monopolar CIS maps. The significantly lower electrode impedance values found for the HiFocus electrode than for the enhanced bipolar arrays for the electrodes used in the monopolar CIS map are predicted by the larger surface area of the electrodes.

The design objective of reduced current requirements for cochlear implant fitting has desirable device aesthetic, safety, and function implications. Reduced power requirements will allow longer battery life if the battery size is held constant, or smaller battery size if the battery life is held constant. Either of these consequences benefits the cochlear implant user. The amount of increase in battery life will depend on the relative power usage of the stimulation pulses as compared to the power usage of the speech processor and transcutaneous signal transmission.

Safe levels of stimulation were documented in early animal studies and were found to be higher than the current levels required for operation of the implant, given the designs that were in use at that time.^{7,8} Because the size of electrodes and the stimulation rate of the processing strategies have changed since these studies, caution is still warranted to continue to ensure safe stimulation limits. No studies have shown adverse effects of stimulation levels used in current electrode designs. Nevertheless, with respect to electrode surface area⁹ and stimulation rate,¹⁰ lower levels of stimulation involve less risk to biological tissue.

Cochlear implant systems stimulate with electric current. As Ohm's law dictates, in order for the system to stimulate with the commanded amount of current, the device must operate within its voltage compliance limits. The upper limit of voltage compliance is inversely proportional to electrode impedance. If a cochlear implant system is operated outside of voltage compliance limits, commanded increases in output current will not result in increases in supplied current. This mode of operation would result in cessation of the growth of loudness with increasing commanded current, and current amplitude modulation clipping that would distort and presumably affect

device performance. The clinical fitting environment of the Clarion cochlear implant, as developed to date, does not allow the clinician to monitor the reaching of voltage compliance limits. Advances in Clarion cochlear implant electrode designs have resulted in lower stimulation levels and lower electrode impedance values. Both outcomes reduce the risk that the cochlear implant system is operating outside of volt-

age compliance.

CONCLUSION

Design modifications for the placement of stimulating electrodes closer to the modiolus in the Advanced Bionics Corporation's Clarion cochlear implant electrode array have resulted in reduced threshold and most comfortable stimulation levels.

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LOCALIZATION OF GLUCOCORTICOID RECEPTORS IN THE MURINE INNER EAR

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We performed an immunohistochemical investigation of the distribution of glucocorticoid receptors (GRs) in the murine inner ear and found that GRs were expressed extensively, but with various degrees of immunoreactivity in different regions. We observed the strongest GR expression in the type III fibrocytes of the spiral ligament. Although the immunoreactivity of the cochlear hair cells and of the vestibular sensory epithelia was weak, the neighboring cochlear supporting cells and the subepithelial regions of the vestibular sensory epithelia were immunostained. Staining for GRs was also positive in the spiral ganglia and vestibular ganglia, as well as in the endolymphatic sac. The role of GRs in the inner ear is discussed.

KEY WORDS — cochlea, endolymphatic sac, immunohistochemistry, spiral ligament, vestibule.

INTRODUCTION

Glucocorticoids are widely prescribed for treatment of various inner ear disorders. However, the detailed mechanisms of glucocorticoid action in the inner ear are yet to be explained. Glucocorticoids are believed to affect levels of various metabolic enzymes and cellular proteins. They enter cells predominantly by passive diffusion and then bind to glucocorticoid receptors (GRs).^{1,2} The GR complex subsequently enters the cell nucleus and affects protein synthesis.

A number of studies have demonstrated GR expression in the inner ear by enzyme-linked immunosorbent assay (ELISA),³⁻⁵ immunohistochemistry,⁶⁻⁹ and in situ hybridization.⁸ However, comprehensive understanding of the distribution of the GRs in the entire inner ear appears to be lacking. We have been studying inner ear immunology in mice.¹⁰⁻¹² Thus, in the present study, we performed GR immunostaining in murine inner ear tissue, believing such a study would inform future basic study of glucocorticoid therapy.

MATERIALS AND METHODS

Six BALB/c mice (male, 6 to 8 weeks of age) were used in this study. All animals were bred in specific pathogen-free conditions and confirmed at sacrifice to be free of middle ear infection. The animals were deeply anesthetized with intraperitoneal pentobarbital sodium and perfused intracardially with physiological saline solution containing 0.1% heparin sodium

followed by 10% phosphate-buffered formalin (pH 7.0). The inferior portion of each bulla was opened rapidly, the stapes was removed, and the round window was perforated. After the temporal bones were immersed in the same fixative for 4 hours, they were decalcified in 0.12 mol/L ethylenediaminetetraacetic acid (EDTA; pH 7.0) for 2 weeks at room temperature. The EDTA solution was changed every other day. The tissues were dehydrated through a graded series of alcohols, cleared in xylene, and embedded in paraffin.

Immunohistochemical Staining Procedures. Horizontal serial sections were cut at 6 μ m from each temporal bone block, and sections from selected regions were deparaffinized, rehydrated, and exposed to 5% normal goat serum in phosphate-buffered saline solution (PBS), then incubated in rabbit anti-GR antibody (Affinity Bioreagents, Golden, Colorado) diluted 1:100 with 1% bovine serum albumin (BSA) in PBS. The polyclonal GR antibody is derived from rabbits that have been immunized with a 22-amino acid (aa 346-367) synthetic peptide, the sequence of which is from the amino terminus of human GR, and it reacts with human, rat, and mouse GRs. The sections were rinsed in PBS and flooded with a 1:200 dilution of biotin-labeled goat anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, California) in 1% BSA-PBS. The sections were rinsed in PBS and incubated with Vectastain Elite ABC reagent (Vector Laboratories). After the sections were rinsed in PBS, binding sites of primary antibody were visualized by development in 0.05%

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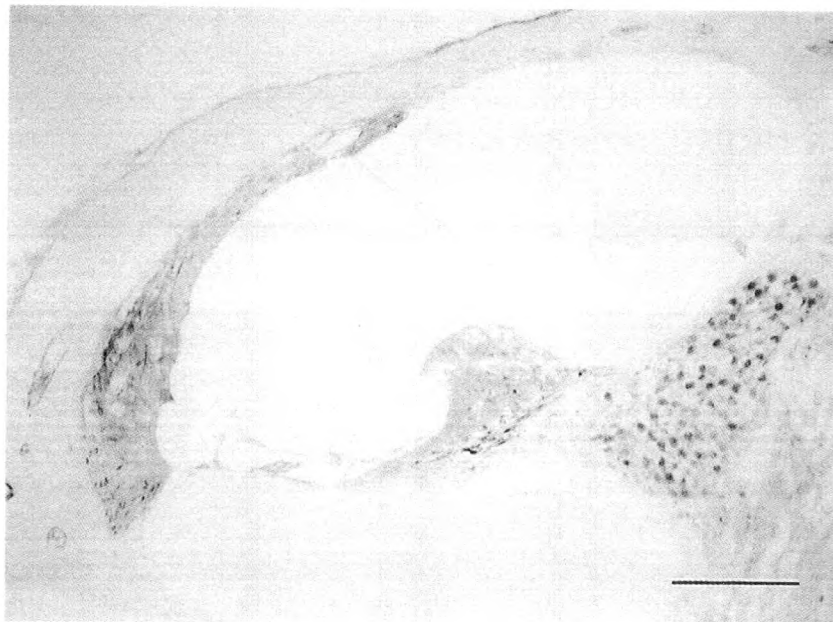


Fig 1. Basal turn of cochlea stained for glucocorticoid receptor (GR). GR expression is detected in lateral wall, organ of Corti, spiral limbus, and spiral ganglion. Bar — 100 μ m.

3,3'-diaminobenzidine–0.01% hydrogen peroxide substrate medium in 0.1 mol/L phosphate buffer. Antibody specificity was confirmed by carrying out experiments as described above in the absence of primary antibody.

Intensity Measurement and Statistical Analysis. Microscopic images were sampled with a Nexus 6800 image processor (Kashiwagi Research Co, Tokyo, Japan) at 256 gradient levels. The average immunostaining intensity was calculated and compared between various inner ear regions. Statistical analyses were performed with the Wilcoxon signed-ranks test. A *p* level of less than .05 was considered statistically significant.

This study was performed in accord with the Law Concerning the Protection and Control of Animals (law No. 105, October 1, 1973, Japan), the Standards Relating to the Care and Custody of Laboratory Animals (notification No. 9, March 27, 1980, Prime Minister's Office, Japan), and the Method for Sacrificing Laboratory Animals (notification No. 40, July 4, 1995, Prime Minister's Office, Japan). The animal use protocol was approved by the Committee on Animal Experiments (CAE) of Oita Medical University (approval No. 9927002, May 7, 1999).

RESULTS

Although GRs were expressed extensively in the inner ear, immunoreactivity varied among the different regions. In the cochlea, GRs were detected in the lateral wall, organ of Corti, spiral limbus, and spiral ganglion (Fig 1). Higher-power images showed the strongest immunoreactivity in the cochlea in the type III fibrocytes of the spiral ligament (SL), which are

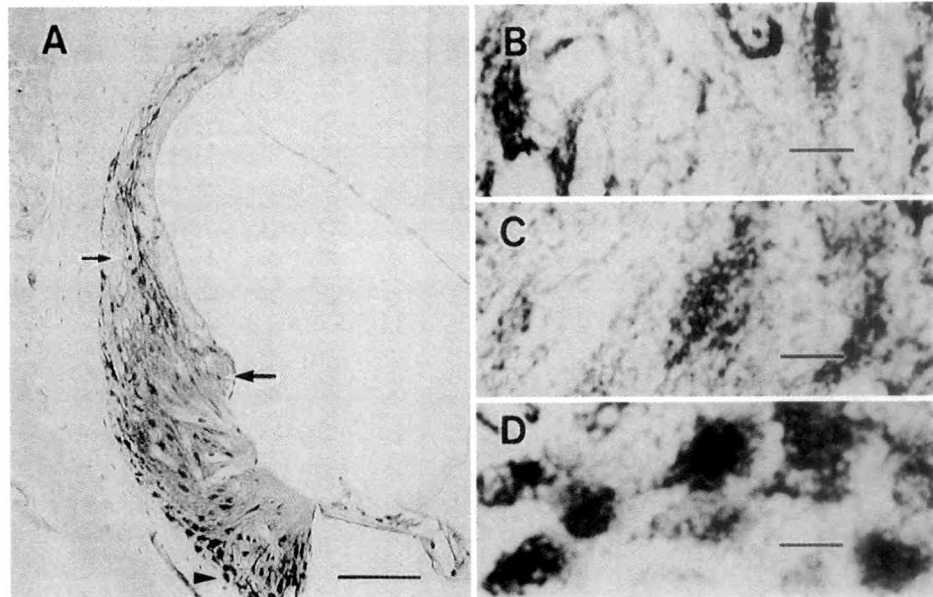
located adjacent to bone in the inferior region of the SL (Fig 2A,D). The SL lateral to the stria vascularis (Fig 2A,B), in which type I fibrocytes are located, and the SL lateral to the spiral prominence epithelium (Fig 2A,C), in which type II fibrocytes are located, showed GR expression that was weaker than that of the type III fibrocytes. The staining intensity in the stria vascularis was almost the same as that in the part of the SL in which type I fibrocytes are located. In the organ of Corti, immunostaining was not obvious in the hair cells, although the neighboring supporting cells were stained (Fig 3).

In the vestibular system, there were absolute staining differences between the sensory epithelium and the subepithelial region. The GR expression in the sensory epithelium of the saccular macula (Fig 4A), utricular macula (Fig 4B), and crista ampullaris (Fig 4C) was obscure, although subepithelial regions were immunostained. It also appeared that the immunoreactivity of the subepithelial region of the crista ampullaris was the strongest immunoreactivity in the vestibular system. Non-sensory epithelium, including dark cells, was also stained. The GR expression in the vestibular ganglion was comparable to that of the spiral ganglion (Fig 5A,B).

In the endolymphatic sac (ES), GRs were stained in the epithelial cells in both the rugose portion (Fig 6A,B) and the distal portion (Fig 6A,C). The subepithelial region was also immunostained.

The average staining intensity for GRs in the inner ear is shown in Fig 7. Although the intensity varied across regions, quantitative measurement showed a definite staining gradient. The GR expression in the SL was strongest in type III fibrocytes and weak-

Fig 2. A) Cochlear lateral wall from basal turn. Bar — 100 μ m. B-D) High-power views of regions in which B) type I (small arrow on A), C) type II (large arrow on A), and D) type III (arrowhead on A) fibrocytes are located. Note that GR expression is strongest in type III fibrocytes. Bars on B-D — 10 μ m.



est in the area in which type I fibrocytes are located, and the difference was significant (type III versus type I, $p < .01$; type III versus type II, $p < .01$; type I versus type II, $p < .01$). The immunoreactivity of the vestibular sensory epithelium was the weakest of that observed in the inner ear (versus saccular macula, $p < .05$; versus utricular macula, $p < .05$; versus crista ampullaris, $p < .01$). There appeared to be an intensity gradient between the subepithelial regions of the vestibular sensory epithelium. The immunostaining was strongest in the crista ampullaris, and the saccular macula was stained more weakly than the utricular macula (crista ampullaris versus utricular macula, $p < .01$; crista ampullaris versus saccular macula, $p < .01$; utricular macula versus saccular macula, $p < .1$).

It should be noted that the staining intensity of the

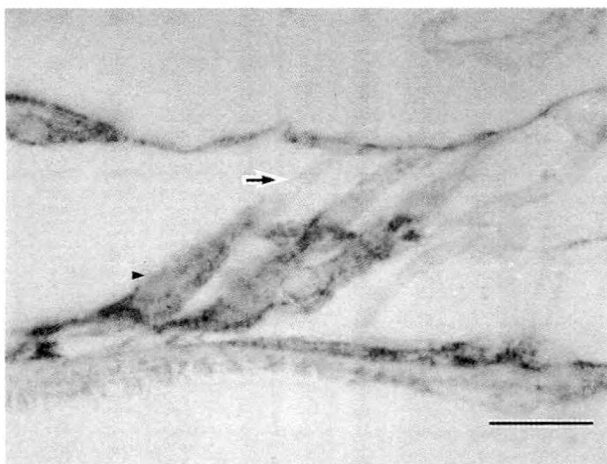


Fig 3. High-power view of organ of Corti. Note that GR immunostaining in cochlear hair cells is not obvious (arrow), although neighboring supporting cells are stained (arrowhead). Bar — 20 μ m.

cochlear hair cells is not well reflected in Fig 7; the entire organ of Corti region, including the hair cells and supporting cells, was intensity-measured. The staining intensity of the cochlear hair cells appeared to be as subtle as that of the vestibular sensory epithelium, as shown in Fig 3. The staining intensity was not measured in the vestibular dark cells, because their area was too small for adequate measurement. We determined subjectively that the staining intensity of the vestibular non-sensory epithelium, including the dark cells, was comparable to that of the stria vascularis and the ES epithelial cells.

DISCUSSION

It has been shown in other tissue systems that the number of GRs in a glucocorticoid target tissue governs the biological response of that tissue to glucocorticoid.^{2,13} It is expected, therefore, that the effect of glucocorticoid on the heterologous inner ear tissues is directly proportional to the number of GRs. Although the immunohistochemical staining in our study was not suitable for detailed quantitative analysis, we were able through image analysis to objectively measure staining intensities. There were regions in which the intensity differed statistically, presumably reflecting differences in the number of GRs.

Previously, Rarey et al^{3,4} isolated inner ear tissues from rats and a human and used ELISA to determine GR levels. The results of their studies, which showed the highest levels of GRs in the SL, are compatible with our immunohistochemical findings. Our study further demonstrated the strongest GR expression in the type III fibrocytes of the SL.

It has recently been speculated that SL fibrocytes play a role in cochlear fluid and ion homeostasis.

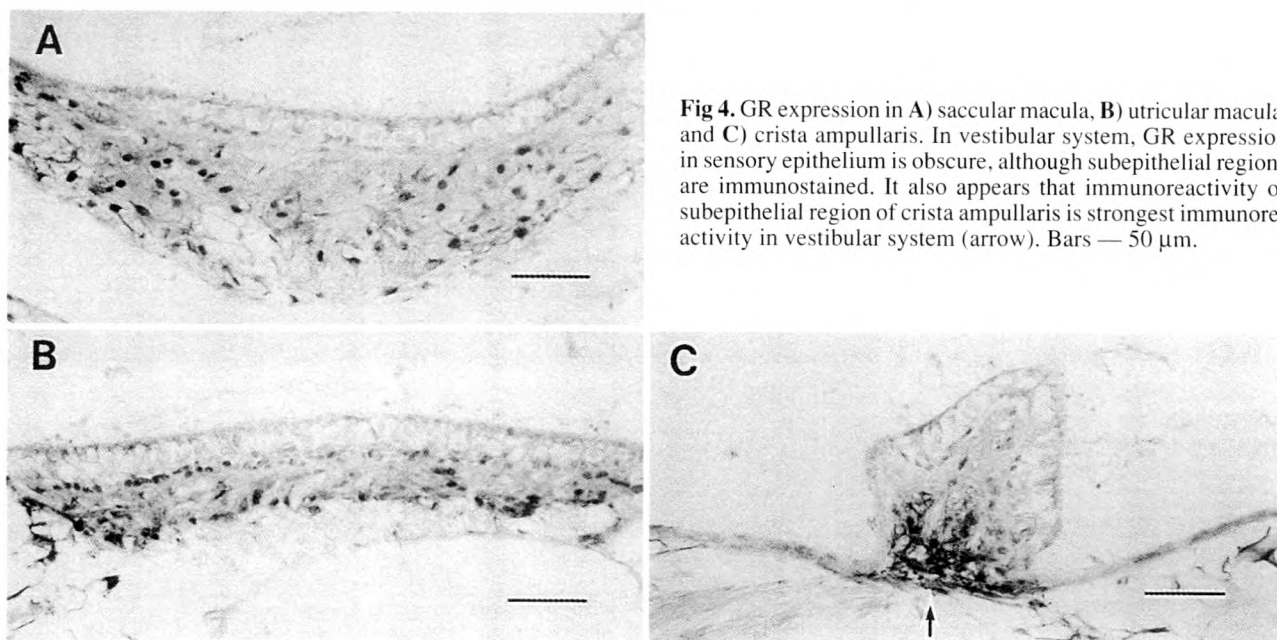


Fig 4. GR expression in **A)** saccular macula, **B)** utricular macula, and **C)** crista ampullaris. In vestibular system, GR expression in sensory epithelium is obscure, although subepithelial regions are immunostained. It also appears that immunoreactivity of subepithelial region of crista ampullaris is strongest immunoreactivity in vestibular system (arrow). Bars — 50 μ m.

This speculation is based on morphological studies that focused on localization of sodium-potassium-adenosinetriphosphatase (Na-K-ATPase)¹⁴⁻¹⁶ and the gap junction protein connexin 26 (Cx26).¹⁷ The Na-K-ATPase in the type II fibrocytes suggests that K^+ ions are taken up from root cells into the SL fibrocytes and that the gap junctions among the SL fibrocytes, including both type I and type II cells, are the route by which K^+ ions move down the concentration gradient into the stria vascularis basal or intermediate cells. It is possible that the Na-K-ATPase-rich strial marginal cells pump the K^+ ions into the cells and that the K^+ ions then flow down a concentration gradient into endolymph.¹⁸ In cochleas with experimentally induced endolymphatic hydrops,¹⁹ labyrinthitis,²⁰ or pneumococcal otitis media,¹⁰ Cx26 immunostaining was reduced in the type I or type II fibrocytes.¹⁹ These findings suggest that the SL fibrocytes

are involved in certain inner ear disorders. The presence of GRs in the SL fibrocytes that we observed suggests that glucocorticoid could be effective in treating such disorders.

We were able to show that GR expression was not prominent in the cochlear hair cells, although neighboring supporting cells in the organ of Corti were positive for GR. This finding suggests that glucocorticoid treatment does not directly affect the cochlear sensory cells. Similarly, vestibular sensory cells are not likely to be direct targets of glucocorticoid, since the immunoreactivity of the vestibular sensory epithelium also appeared subtle, despite the positive GR staining of the subepithelial regions.

According to one of the ELISA studies by Rarey et al,³ rat inner ear tissues have more abundant GRs in the cochlea than in the vestibule. Rarey et al hy-

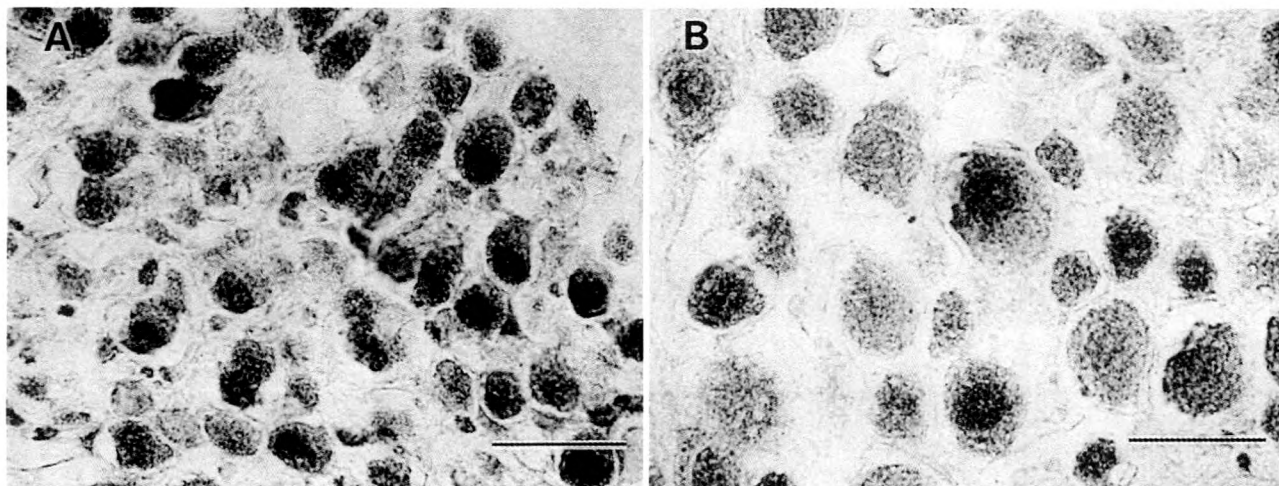
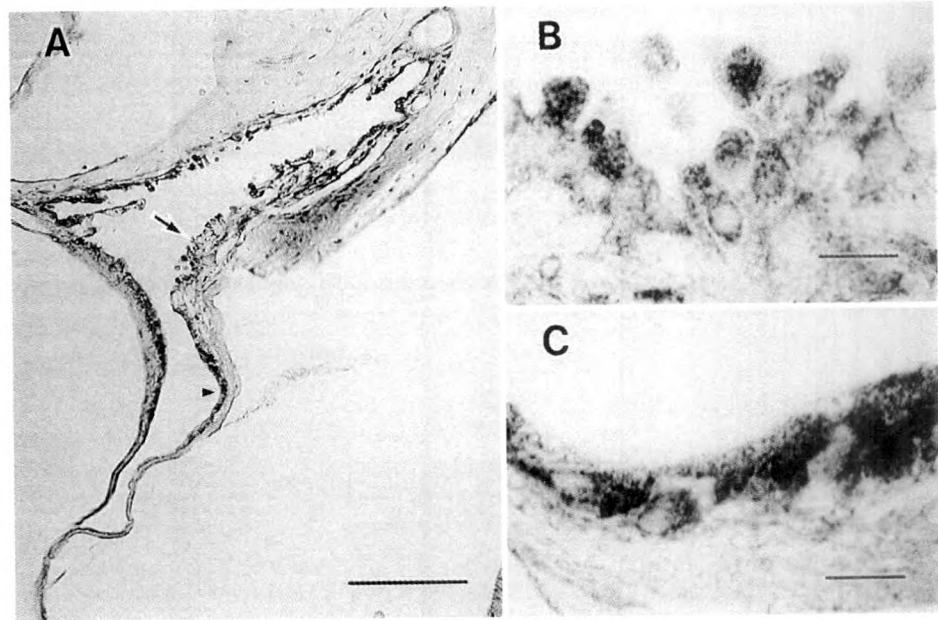


Fig 5. **A)** Spiral ganglion and **B)** vestibular ganglion, which show comparable immunostaining for GR. Bars — 20 μ m.

Fig 6. A) GR expression in endolymphatic sac. GRs were immunostained in both rugose portion (arrow) and distal portion (arrowhead). Bar — 100 μ m. B,C) High-power views of B) rugose portion and C) distal portion. Bars in B and C — 10 μ m.



pothesized that cochlear tissues are more biologically responsive to circulating glucocorticoid than are tissues of the vestibular membranous labyrinth. However, our study showed that GR expression was low in both the cochlear hair cells and the vestibular sensory epithelia and that the GRs stained as strongly in the vestibular ganglia as in the spiral ganglia — a finding that makes it reasonable to speculate that these two counterparts of the cochlea and the vestibule are comparably responsive to circulating glucocorticoid. Since type III fibrocytes of the SL have the strongest GR expression, one can speculate that they are the most biologically responsive to gluco-

corticoid. The functional role of the type III fibrocytes is not known, and it needs to be clarified.

Unlike the function of the cochlear fibrocytes, the functional significance of the vestibular fibrocytes is not clear.²¹ However, the GR immunostaining pattern we observed in the vestibule is interesting in light of the results of the ELISA study by Rarey and Curtis.⁴ They examined human inner ear tissue and reported that the crista ampullaris contained prominent levels of GR and that the GR levels of the saccular macula were low. Our study showed a similar intensity gradient among subepithelial regions of the vestibular sensory epithelium. The presence of GRs in a specialized region might reflect the differentiated function of the subepithelial region of the vestibule.

It has also been shown by ELISA that GRs are expressed in the ES,⁴ and our immunohistochemical findings confirmed their presence. We have further demonstrated that GRs are expressed in both the epithelial and subepithelial regions of the ES. The epithelial cells in rugose and distal ES portions are reported to be Na-K-ATPase-immunopositive, but less so than stria vascularis cells, and ES epithelial cells are also reported to be positive for calcium-adenosinetriphosphatase (Ca-ATPase) and calcium-binding proteins.¹⁵ Although our study showed that the ES and the stria vascularis are similar in terms of GR expression, the lower levels of Na-K-ATPase and the coexistence of Ca-ATPase and calcium-binding proteins in the ES epithelial cells indicate that these cells have a distinctive role in ion transport that differs from that of the cells of the stria vascularis. A possible immunologic role for the ES has also been reported. Using secondary keyhole limpet hemocya-

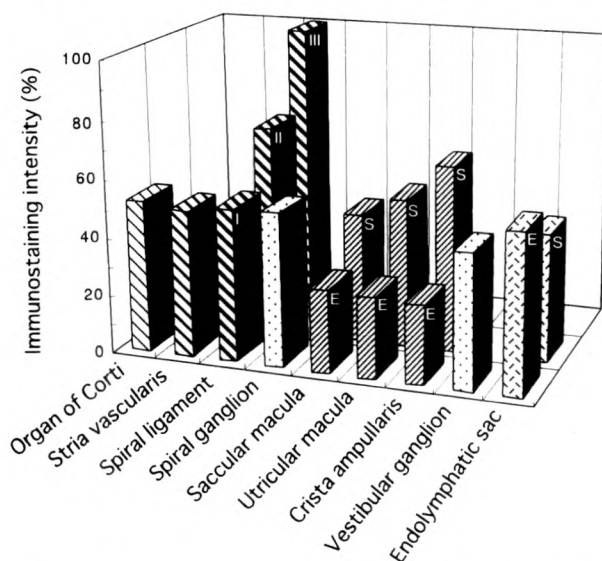


Fig 7. Average immunostaining intensity for GRs in inner ear. Intensity in spiral ligament type III fibrocytes is presented as 100%. I — type I fibrocyte region; II — type II fibrocyte region; III — type III fibrocyte region; E — epithelial region; S — subepithelial region.

nin challenge in the ES, Tomiyama²² induced endolymphatic hydrops in guinea pigs and concluded that endolymphatic hydrops is induced by the immune response of the ES. Furthermore, a study of a patient with active Meniere's disease²³ showed signs of focal inflammation with intraepithelial invasion by mononuclear cells in the ES. If such "endolymphatic sacitis" is related to the pathogenesis of Meniere's disease, GR expression in the ES could explain the effectiveness of glucocorticoid therapy in patients

with this disorder.

CONCLUSION

We demonstrated that GR expression is heterogeneous in the inner ear. We conclude that glucocorticoid should have different effects in the different regions of the inner ear and that precise determination of the impaired regions in various inner ear disorders might make it possible to refine the indications for glucocorticoid therapy.

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INTERNET RESOURCES AVAILABLE TO OTOLARYNGOLOGISTS

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During recent years, the Internet has evolved into the largest computer network in the world, allowing access to vast amounts of information and services. Medical information is increasingly available to both patients and professionals, and ever more biomedical resources are becoming available on-line to assist in research, clinical medicine, and education. The Internet has always provided useful resources to otolaryngologists, implemented at various academic departments and national organizations or by specialists or specific medical web sites offering technical, scientific, and biomedical information. The purpose of this article is to provide as complete a list as possible of the World Wide Web sites accessible through the Internet that are of interest to otolaryngologists. It summarizes different types of resources available, including educational material, audiology sites, outcomes research, discussion lists, research laboratories, publications, medical libraries, news and medical conferences, organizations, academic departments, otolaryngological and medical resources, medical and surgical equipment and suppliers, and miscellaneous other sites of interest to otolaryngologists. This review is intended to present the wealth of the accessible information on the Internet and to promote the use of the network to otolaryngologists who do not have extensive experience in computers or telecommunications.

KEY WORDS — computer communication networks, Internet, otolaryngology.

During recent years, the Internet has expanded from being a tool of the scientific community and computer experts to become the most-promoted and most rapidly growing worldwide technology.^{1,2} Electronic mail is used by millions of people connected to the Internet because of its inherent advantages versus surface mail, including immediate transmission and the ability to transfer computer files including images and sound. The World Wide Web, the other most common use of the Internet, is a universal network in which many governments, nonprofit organizations, corporations, companies, scientific institutions, and universities, as well as individuals, maintain home pages including text and other media. All of these home pages are connected through links to other sites with relevant content, which in turn are connected to more sites, creating thus a vast network interconnected by multiple links and known as the World Wide Web. Communication among people around the world may be performed on the Internet, either through immediate conversation or through mailing lists and discussion groups about specific topics.³ The usefulness of this possibility in the field of medicine is obvious, since specialists and general practitioners may communicate with experts from various countries and obtain information about diagnostic and therapeutic issues. Furthermore, voice and image transfer is made possible by modern multimedia technology, and video conferences may be held.

The Internet has always provided useful resources to otolaryngologists, but in recent years the breadth, content, and scope of the Internet-based resources have increased remarkably.⁴⁻⁷ Many academic departments, otolaryngology associations, scientific journals, research laboratories, medical libraries, and otolaryngologists maintain interesting sites with information about their activities on the World Wide Web.⁸⁻¹¹ Sites useful to specialists and patients may be found on the Web, providing educational material, statistical data, and all of the modern developments in this specialty.¹² Literature searching in medical literature databases such as Medline, a database maintained by the National Library of Medicine in the United States, is a Web-based free service called PubMed and is one of the main reasons that most physicians use the Internet.¹³ Furthermore, mailing lists on specific topics or subspecialties of otolaryngology are maintained by various moderators,^{14,15} and useful commercial sites of medical and surgical equipment are available.

Despite the modern computer and telecommunications technology used, the Internet presents nevertheless some definite disadvantages. The first is reduced security in the process of transferring data between various sites, which poses special ethical and legal issues regarding management of patients' data. Of course, an elementary level of security al-

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ready exists, but violation will still be possible until advanced encryption algorithms are available. The second disadvantage is the slow transfer rate of data such as graphics, video images, and voice. This transfer is expected to be improved in the near future by increased bandwidth of communication channels between Internet providers and users and by new satellite technology. Finally, the vast amount of data available on the World Wide Web makes navigation across the relevant sites and searching of information about a topic quite difficult. This problem may be confronted by using the search engines freely available on the Internet, but the obtained information is quite often inaccurate and may contain many useless links. On the other hand, some interesting sites on the Web cannot be easily accessed. One possible solution is following the links from one site to other relevant sites in order to find the necessary information, but this is a time-consuming process. Another way is visiting special sites on the World Wide Web, including long lists of otolaryngology sites such as those maintained by some academic departments, but many of them are incomplete, contain old links that do not respond anymore, or do not include a universally oriented list, but rather, emphasize local resources. Since many interesting otolaryngology sites exist all over the world, the aim of this report is to present an exhaustive list of these resources by updating and completing previously published lists.¹⁶⁻²⁰ The sites are classified in order to assist the physician in finding immediately the necessary information, instead of navigating across various links offered by on-line search engines. Another aim of this report is to display the wealth of information of interest to otolaryngologists that is available on the Internet, in order to promote use of the Internet by otolaryngology specialists who are not experienced in use of the medium.

The uniform resource locator (URL) addresses of all of the otolaryngology sites on the Internet have been found and were subdivided into categories. However, the reader should be aware that the addresses of web sites can sometimes change without notice, or a site might be temporarily or permanently not responding, possibly because of a lack of funds. The main categories we have used are educational material, audiology sites, outcomes research, discussions, research laboratories, publications, medical libraries, news and medical conferences, organizations, academic departments, otolaryngology and medical resources, medical and surgical equipment and suppliers, and, finally, miscellaneous other sites of interest to otolaryngologists (see Table). Most information was collected by navigating across the Internet, starting from sites containing lists of otolaryngology sites and further accessing these sites and the various links

connected to them. Each site was visited at least twice during the past year in order to confirm that it was active and responding properly. Nonresponding links were excluded from our lists. Special effort was taken to include all relevant sites and to compose a complete, universal list that presents all of the information on the Internet of interest to otolaryngologists.

The 1st category of URL addresses is that of educational sites, divided into patient education (69 sites), general otolaryngology (47 sites), otology-neurotology (59 sites), rhinology (93 sites), laryngology (10 sites), voice disorders (18 sites), pediatric otolaryngology (17 sites), facial plastic and reconstructive surgery (9 sites), head and neck cancer (14 sites), and pathology and imaging sites (40 sites). The section on rhinology is subdivided into 5 parts: allergy, chemical senses, endoscopy and operative techniques, rhinologic clinics and research centers, and miscellaneous rhinology sites. The 2nd category includes audiology links, which are divided into 3 main subcategories, the 1st including audiological scientific societies and support organizations (52 sites), the 2nd including audiology centers (88 sites), and the 3rd consisting of miscellaneous audiology sites (207 sites) such as acoustics, audiology resources, cochlear implants, hearing loss and deafness resources, hearing aids, newborn hearing screening, noise-induced hearing loss, otoacoustic emissions, speech, tinnitus, vestibular diseases, and various other sites.

The 3rd category includes outcomes research sites (18 sites), either sites of general medical interest or specific otolaryngology sites, and the 4th category includes discussion groups and mailing lists about specific topics in otolaryngology (50 sites) maintained by specialists in each subject. The 5th category includes research laboratories (154 sites), mainly active in cochlear and vestibular research, but some others involved in acoustics and other interesting fields of research are also included, either affiliated with universities or not. Besides these specialized laboratories, some other institutes of patient care and education are included as well. The 6th category consists of 2 separate groups. The 1st (108 sites) includes the sites of otolaryngology journals and some other medical journals of general medical interest. The 2nd group (24 sites) includes publishing companies that specialize in otolaryngology books, journals, and CD-ROMs.

The 7th category includes selected home pages of medical libraries and some collective library links (80 sites). The 8th category includes sites on otolaryngology news and medical conferences (20 sites). The 9th category includes the sites of professional societies, organizations, and associations (171 sites). They

INTERNET RESOURCES AVAILABLE TO OTOLARYNGOLOGISTS*

Main Categories	Subcategories
Education links	Patient education General otolaryngology Otology-neurotology Rhinology (allergy, chemical senses, endoscopy—operative techniques, rhinologic clinics and research centers, various) Laryngology Voice disorders Pediatric otolaryngology Facial plastic and reconstructive surgery Head and neck cancer Pathology images
Audiology	Scientific societies and support organizations Audiology centers Miscellaneous (acoustics, audiology resources, cochlear implants, hearing loss—deafness, hearing aids, newborn hearing screening, noise-induced hearing loss, otoacoustic emissions, speech, tinnitus, vestibular diseases, various)
Outcomes research	General Otolaryngological
Discussion and Usenet groups	
Research laboratories	
Publications	Journals Medical publishing companies
Medical libraries	
News and medical conferences	
Organizations and societies	American otolaryngological societies International otolaryngological societies Other organizations Support organizations
Universities and academic programs	United States, Canada, Scandinavia, Germany, United Kingdom, Italy, other European countries, Japan, other countries of Asia, Australia, South America
Otolaryngology resources	
Other medical resources	
Medical and surgical equipment and suppliers	
Miscellaneous	

*Full listings available at <http://www.annals.com/resources>

are classified into 4 groups, the 1st including American sites, the 2nd the international ones, and the 3rd including organizations with an emphasis on topics of general interest to otolaryngologists, such as sleep research, thyroid disorders, and acoustics. The 4th group includes support organizations.

The 10th category includes academic departments of otolaryngology, classified according to their location (245 sites). The subgroups include the United States, Canada, Scandinavia, Germany, the United Kingdom, Italy, other European countries, Japan, other countries of Asia, Australia, and South America. The 11th and 12th categories include otolaryngology resource lists (50 sites) and general medical resources (40 sites), respectively. The 13th category includes the sites of suppliers of medical and surgical equipment and hearing aids and various other companies (101 sites). Finally, the 14th category contains miscellaneous links (25 sites) of interest to otolaryngolo-

gists.

The educational sites are perhaps the most interesting of the otolaryngology resources. Some of them are for patients, who may quickly obtain the latest information on late-breaking research, therapeutic modalities, support groups, and individual coping strategies. Most of the other links are professional sites with valuable information on otolaryngology topics. Insights into various otolaryngological diseases are offered, and interesting sites about temporal bone dissection, digital anatomy, surgical videos, 3-dimensional images, and computer applications cover all aspects of the specialty, using advanced multimedia technology. Some topics of other specialties, but of interest to the otolaryngologist, such as plastic surgery, neurology, and neurosurgery, are also included.

Audiology links include information about most organizations, societies, and associations worldwide.

The information provided on such web sites is usually a brief history of the organization, information about membership, announcements of meetings and conferences sponsored by the organization, a directory of members and board members, and, sometimes, interesting educational information and links to relevant sites. Audiology centers and institutes active in research in the field of hearing and vestibular function, as well as clinical centers for patient care, diagnosis, and treatment, are also included. Finally, various sites on audiology topics are listed, with interesting information about all modern developments in audiology, such as cochlear implants and digital hearing aids, deaf resources, research issues, medicolegal databases about noise pollution, speech links, and tinnitus and vertigo resources, with special sites about Meniere's disease. Many sites from the modern fields of otoacoustic emissions and newborn hearing screening programs are also included.

Outcomes research sites are mainly based in the United States and contain useful information about projects in progress on common otolaryngological disorders, but some other sites of general medical interest are also included. Discussion groups and mailing lists maintained by various specialists or academic departments offer valuable information to other professionals or patients about common diseases. These sites can aid in interaction between the referring and consulting physicians and in medical education. Besides the English sites, some German, Italian, French, and Spanish sites are included as well.

Laboratories active in research, either affiliated with universities or not, are included, presenting usually their activities and their past and present projects. The main fields of research include hearing and vestibular function, acoustics, cochlear implants and newer technologies, simulation projects, genetics, psychoacoustics, computer applications, and neurosciences.

Most otolaryngology journals are represented well on the Internet by sites including information about the publishing companies, instructions to authors, information for subscribers, and prices. Abstracts and lists of contents of all of the past issues are usually included, and in a few cases, full-text articles accompanied by color images, graphics, and videos may be obtained. A few purely on-line journals are also presented, and some journals of general medical interest are included as well. Publishing companies that specialize in otolaryngology products present information about textbooks, CD-ROMs, and other educational material with on-line catalogs. On-line orders by e-mail are usually possible, and new editions are announced and may even be ordered in advance.

Medical libraries all over the world maintain sites in which information about their services is included, as well as lists of their books and medical journals. Because of the large number of relevant sites, only several selected sites are included here. The section on otolaryngology news and conferences has few sites, but may be very useful to busy professionals, since new developments in the specialty are summarized and information is given about upcoming medical conferences, congresses, and seminars.

The group of professional and support organizations, societies, and associations includes many web sites with information about the objectives and rules of the organization, announcements, educational material, and membership information. Most of these organizations are located in the United States, but many others across the world are represented by excellent web sites. Valuable information also may be obtained by the otolaryngologist, not only from the strictly otolaryngological organizations, but from others concerned with associated topics, such as sleep research, thyroid disorders, computer science, and other medical specialties, including plastic surgery, general surgery, pediatrics, and pathology. Sites of support organizations, addressed mainly to patients, are an excellent source of information about various diseases, with head and neck cancer possessing a prominent place among them.

Academic departments of otolaryngology from all the continents, but again with the main contribution from the United States, are represented with mostly excellent web sites as part of the home page of the university. The information provided includes faculty profiles and directories, residency and educational programs, postgraduate courses, clinical activities and research projects, seminars and conferences, and general information about the city and state of the university's location. Finally, in many sites, topics of broad interest, such as handbooks, databases, specific otolaryngology topics, and links to other sites are included.

Collective lists of otolaryngology and other medical resources on the Internet are presented separately, and some of them are a good start for navigation by using the links of each site. However, most of them are very short, and some excellent otolaryngology sites are omitted. Many of the lists have not been revised during recent months or years, and include sites that no longer exist or whose URLs have changed.

Another group of interest to the professional is the list of sites of medical and surgical equipment and suppliers. These are located mainly in the United States and Europe, providing on-line catalogs, information about each product, and on-line technical

assistance and other services. All the major companies that provide surgical and laboratory equipment, hearing aids, cochlear implants, and other relevant products are represented on the Internet.

Finally, miscellaneous links of interest to otolaryngologists or patients are presented, such as lists of specialists in various regions, medical information, and databases. A special mention must be given to PubMed, a free service available on the Internet from various web sites, providing the option of literature searching of the journals indexed in the National Library of Medicine by using various keys, such as the author's name, the title, the subject, the journal, or

key words.

In conclusion, otolaryngology resources on the Internet have greatly expanded during recent years, and the aim of this report is to present the wealth of the available information to otolaryngology specialists who are not quite familiar with this medium and furthermore, to promote the usefulness of the Internet. It was intended, also, to assist professionals to locate any information easily on the World Wide Web, and for that reason an exhaustive list of all web sites of interest to otolaryngologists is presented on the web site of this journal,²¹ the main categories of which appear in the Table presented here.

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BOOK REVIEWS

Minimally Invasive Surgery of the Head, Neck, and Cranial Base

Phillip A. Wackym, Dale H. Rice, and Steven D. Schaefer, editors. Hard cover, illustrated, indexed, 576 pages, 2001. Lippincott Williams & Wilkins, Philadelphia, Pa, \$225.

Purpose: To provide a comprehensive book that addresses the advances, advantages, and limitations of endoscopic techniques in all aspects of otolaryngology–head and neck surgery.

Contents: There are 67 contributing authors who have written 45 chapters. The book is divided into 5 sections ranging from general principles (optics, digital image processing, stereotactic surgery, and lasers) to subspecialty applications of endoscopic techniques. Some of the subspecialties have included extensive anatomic and physiological discussions.

Attributes and Limitations: Endoscopic anatomy simply describes known anatomic details observed under different viewing conditions. Structures seen through the scope are viewed at an angle different from that of the operating microscope. As a result, the relative sizes of the structures seen through an endoscope and a microscope are different. Structures already passed by the front lens lie outside its field of view and, particularly with flexible scopes, are no longer seen.

True endoscopic surgery with improved existing instruments and newly developed technologies, eg, lasers for cutting and drilling, is presented in this well-written and color-illustrated volume. Most of the authors give a very comprehensive coverage to all aspects of the subject, especially operative techniques.

If the book had focused on the detailed methods of techniques of endoscopy in the practice of otolaryngology–head and neck surgery, it would have been a unique volume. Instead, a run of 75 continuous pages of anatomy, pathophysiology of paranasal sinuses, and imaging techniques gives information that is found in other sources and not necessary herein. The intraoperative color endoscopic photographs, gray-scale photographs, patient positioning, and equipment photographs, along with interspersing of historical perspectives, provide accessibility to delightful reading.

Applicability: The endoscope augments rather than replaces the binocular microscope. This philosophy is emphasized in the book. In the ear, it allows the surgeon to see around corners, as in the sinus tympani and the anterior extension of the epitympanum. A financial and time investment to learn its applications is necessary. This book is of great value to those who want to broaden their technique.

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Otolaryngology: The Essentials

Allen M. Seiden, Thomas A. Tami, Myles M. Pensak, Robin T. Cotton, and Jack L. Gluckman, editors. Soft cover, illustrated, indexed, 700 pages, 2001. Thieme, New York, NY, \$69.

Purpose: To provide basic science and clinical information in such a fashion that the reader may remember or retain the essential information. Most sections put emphasis on the reading of the text, offering unique features such as shaded rectangles (pearls) and boxed areas (controversies) to enliven and increase appreciation of the information. Also set off are “special considerations” (special information for additional emphasis) and “pitfalls” (pointing out potentially misleading information and situations that might lead to incorrect diagnosis).

Contents: All anatomic sites of the ear, nose, throat, head, and neck are included, along with sections on skin lesions, flap reconstruction, syndromes and eponyms, and aesthetic surgery of the face.

Attributes: The authors have presented a concise review of the majority of clinically significant information. The highlighted vignettes within the body of the text draw additional attention to important points that would be possibly overlooked or buried in a more cumbersome text.

Limitations: Connections made between anatomic sites, clinical test results, and diagnoses via a clear explanation of the physiology involved are, at best, incomplete. For example, the importance of the role of outer hair cells in audition and the diagnostic use of otoacoustic emissions are not afforded sufficient attention. Also, the related subject of auditory dyssynchrony (neuropathy) is entirely omitted. (This subject is commonly referred to in the literature today.)

Applicability: This text is a fitting review source for otolaryngologists, residents, or any clinician involved in the specialty with an interest in quick access to clinically applicable information. This is not an in-depth book, yet it provides the young otolaryngologist essential basic information in such a way that it may be retained.

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INNOVATIONS IN PHONOSURGERY

**VOICE AND TREATMENT OUTCOME FROM PHONOSURGICAL
MANAGEMENT OF EARLY GLOTTIC CANCER**

STEVEN M. ZEITELS, MD
ROBERT E. HILLMAN, PhD
RAMON A. FRANCO, MD
GLENN W. BUNTING, MS

**PHONOMICROSURGERY IN SINGERS AND PERFORMING ARTISTS:
TREATMENT OUTCOMES, MANAGEMENT THEORIES,
AND FUTURE DIRECTIONS**

STEVEN M. ZEITELS, MD
ROBERT E. HILLMAN, PhD
ROSEMARY DESLOGE, MD
MARCELLO MAURI, MD
PATRICIA B. DOYLE, MA

ANNALS
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DEDICATION

This work is dedicated to Steven D. Gray, MD (1953-2002), whose pioneering efforts in the development of molecular biology of the larynx provided the inspiration and foundation for many concepts contained herein. His scientific vision has enabled laryngologists to construct directions for future discovery and strategies to solve yet-unanswered questions.

This work was supported in part by the Demetre Souretis Research Fund.

INTRODUCTION

Laryngology developed subsequent to the presentation “Observations on the Human Voice” (1855), by the opera maestro Manuel Garcia. Since then, there has been an inextricable linkage between laryngology and human voice production. A primary driver for the development of 19th-century laryngology was mirror-guided management of laryngeal airway obstruction, primarily from infectious diseases of the upper aerodigestive tract. This emphasis continued into the early 20th century, when Kirstein, Killian, and Jackson popularized direct endolaryngeal surgery. However, the introduction of mass-produced cigarettes around 1910 led to a sharp increase in the incidence of laryngeal cancer, which became a critical focus of the specialty. Although procedures to enhance the voice have been described since the inception of laryngology, the greatest emphasis has appropriately been on airway safety, swallowing function, and the treatment of cancer. Many important problems in these areas have been solved by previous generations of laryngologists. Phonosurgery has emerged at the forefront of laryngology because of the relatively recent convergence of scientific initiatives in voice disorder physiology and advances in minimally invasive technology.

The investigations reported in this publication represent a decade of phonosurgical development with disparate patient populations and clinical problems. It was our impression that the critical algorithmic assessment and surgical planning inherent to oncological management could serve those who care for singers with lesions. Conversely, we thought that the finesse and ultraprecise techniques required for successful surgical treatment of vocalists could potentially benefit surgeons who manage early glottic cancer.

In the first manuscript, entitled “Voice and Treat-

ment Outcome From Phonosurgical Management of Early Glottic Cancer,” techniques for phonosurgical excision and reconstruction are described along with outcome data on oncological efficacy as well as vocal function. Endoscopic excision and radiotherapy are similarly effective in achieving high cure rates (around 90%) for early glottic cancer. Therefore, a primary metric to judge the success of treatment is the vocal outcome. Resection methods to enhance voice preservation, along with new reconstructive techniques, enable a majority of patients with early unilateral glottic cancer to achieve a normal conversational-level voice. We hope that this investigation will catalyze a reappraisal of treatment paradigms for early glottic cancer, since a majority of patients are managed with irradiation, which is an invaluable single-use cancer treatment.

In the second manuscript, “Phonomicrosurgery in Singers and Performing Artists: Treatment Outcomes, Management Theories, and Future Directions,” new concepts of vocal fold vibration are introduced in regard to physiological function and dysfunction. The clinical theories are based in part on Steven Gray’s seminal investigations into the layered microstructure of the vocal fold. It is our hope that concepts that evolved from the clinical study of the anatomy and function of vocalists will serve to assist otolaryngologists treating any patient with lesions of the vocal fold phonatory edge. The findings of both investigations establish that loss of epithelial pliability is a primary determinant of dysphonia, and we believe that future initiatives to design a biosubstitute for the superficial lamina propria will be of benefit to a majority of patients with hoarseness. Phonosurgery is a dynamic subspecialty in otolaryngology with a rich heritage and an exciting future.

VOICE AND TREATMENT OUTCOME FROM PHONOSURGICAL MANAGEMENT OF EARLY GLOTTIC CANCER

STEVEN M. ZEITELS, MD

RAMON A. FRANCO, MD

ROBERT E. HILLMAN, PhD

GLENN W. BUNTING, MS

BOSTON, MASSACHUSETTS

Phonosurgical management of early glottic cancer has evolved considerably, but objective vocal outcome data are sparse. A prospective clinical trial was done on 32 patients with unilateral cancer (T1a in 28 and T2a in 4) who underwent ultranarrow-margin resection; 15 had resection superficial to the vocal ligament, and 17 deep to it. The subepithelial infusion technique facilitated selection of these patients for the appropriate procedure. All are cancer-free without radiotherapy or open surgery. Involvement of the anterior commissure (22/32) or the vocal process (15/32) of the arytenoid cartilage did not influence local control. Nine of 17 patients had resection of paraglottic musculature, and all underwent medialization reconstruction by lipoinjection and/or Gore-Tex laryngoplasty. Eight of the 17 had resections deep to the vocal ligament, but without vocalis muscle, and 1 of the 8 underwent medialization. Posttreatment vocal function measures were obtained for all patients. A clear majority of the patients displayed normal values for average fundamental frequency (72%) during connected speech, and normal noise-to-harmonics ratio (75%) and average glottal airflow (91%) measures during sustained vowels. Smaller majorities of patients displayed normal values for average sound pressure level (SPL; 59%) during connected speech and for maximum ranges for fundamental frequency (56%) and SPL (59%). Fewer than half of the patients displayed normal values for sustained vowel measures of jitter (45%), shimmer (22%), and maximum phonation time (34%). Almost all patients had elevated subglottal pressures and reduced values for the ratio of SPL to subglottal pressure (vocal efficiency). There were significant improvements in a majority of patients for most vocal function measures after medialization reconstruction. Normal or near-normal conversation-level voices were achieved in most cases, regardless of the disease depth, by utilization of a spectrum of resection and reconstruction options. These favorable results are based on establishing aerodynamic glottal competency and preserving the layered microstructure of noncancerous glottal tissue.

KEY WORDS — cancer, dysphonia, fat injection, glottis, hoarseness, laryngoplasty, lipoinjection, phonomicrosurgery, phonosurgery, stroboscopy, thyroplasty, vocal cord, vocal fold, voice.

INTRODUCTION

Solis-Cohen was probably the first to cure an early glottic cancer and did so by means of a transcervical partial laryngectomy in 1867.^{1,2} In 1886, Fraenkel³ reported the first successful transoral (mirror-guided) treatment of an early vocal fold cancer. After Kirstein^{4,5} popularized direct laryngoscopy and Killian⁶ established the advantages of suspension laryngoscopy, Lynch⁷ published the first series of direct laryngoscopic early glottic cancer resections. Because of the advanced skill level that was required for effective laryngoscopic management, open surgery and radiotherapy became the mainstays of treatment and remained so through the 20th century.

During the past 25 years, there was some expansion of the role of endoscopic management with the introduction of the CO₂ laser by Jako, Strong, and Vaughan.⁸⁻¹² Subsequent to describing the body-cover theory of vocal fold vibration,¹³ Hirano reported use of the CO₂ laser for varying depths of resection

to successfully narrow cancer margins.¹⁴ Then, Zeitels et al¹⁵ described the subepithelial infusion technique, which enhanced this concept further with both cold instrument and laser microflap resection.¹⁶ Convergence of phonosurgical theories of voice production with evolving microsurgical approaches culminated in the phonomicrosurgical technique.¹⁷ The initial pilot series of patients who underwent resection by this technique with ultranarrow margins was reported in 1996.¹⁸

Presently, there seems to be frequent discussion about whether the ideal management of early glottic cancer is radiotherapy or CO₂ laser treatment. This perspective excludes cold instrument resection, which has phonatory advantages with superficial lesions,^{17,19} as well as new tissue manipulation paradigms. Despite the fact the CO₂ laser is essentially functioning as an efficient scalpel, advocacy of laser treatment indirectly implies that there is enhanced oncological effectiveness from this nonionizing radiation. To

From the Department of Otolaryngology, Harvard Medical School (Zeitels, Hillman, Franco), and the Division of Laryngology (Zeitels, Franco) and the Voice and Speech Laboratory (Zeitels, Hillman, Bunting), Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

Presented at the meeting of the American Laryngological Association, Palm Desert, California, May 12-13, 2001.

CORRESPONDENCE — Steven M. Zeitels, MD, Division of Laryngology, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114.

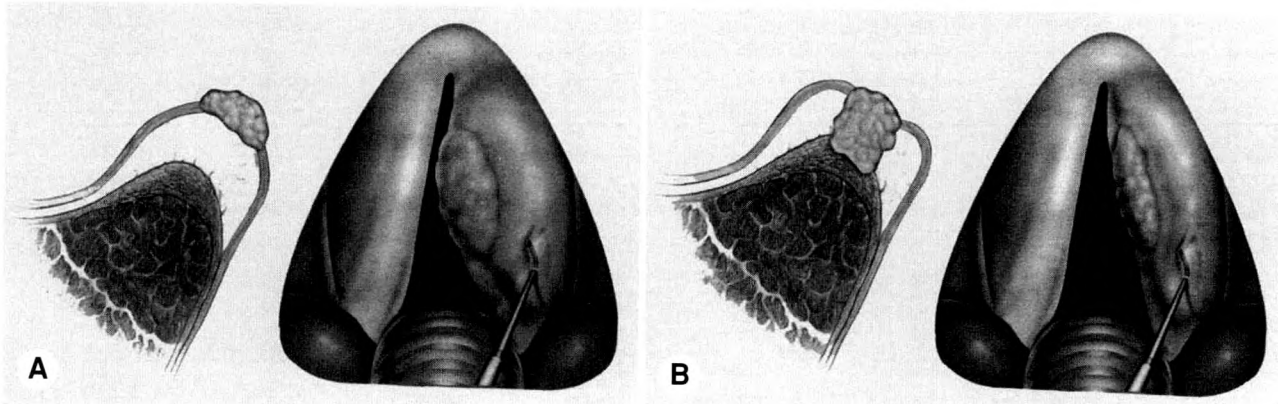


Fig 1. Diagrammatic representations of subepithelial infusion in which there is **A)** no invasion and **B)** invasion of epithelial lesion into vocal ligament. (Reprinted with permission.³⁵)

avoid confusion, it is suggested that general terms such as endoscopic, endolaryngeal, phonomicrosurgical, or transoral resection be used with the additional qualifier of CO₂ laser or cold instrument, etc.

In the past several years, endoscopic medialization of the postresection healed neocord from these cancer resections has been done by means of lipoinjection²⁰ and/or Gore-Tex²¹ laryngoplasty²² to enhance the vocal outcome. Innovative phonosurgical management of early glottic cancer comprises employing ultranarrow margins during the resection and subsequently medializing the neocord, if it is excavated and associated with aerodynamic incompetence. This investigation examined the vocal outcome from the aforementioned combined excision and reconstruction method, as well as the oncological efficacy of the ultranarrow-margin resection technique.

METHODS

Phonomicrosurgical Resection Technique. Wide exposure²³⁻²⁷ of the glottal cancer field enhances endoscopic resections and is achieved with the triangular lancet arch-shaped universal modular glottis-

scope.^{27,28} Placement of the largest examining speculum requires the use of a true suspension gallows^{6,29,30} and results in elevated vector suspension.^{17,25} External counterpressure and internal distention also enhance exposure.^{23,25,26,31} As necessary, portions of the lower supraglottis are resected with the CO₂ laser to facilitate exposure of the cephalad surface of the glottis.³²⁻³⁴

Subepithelial saline-epinephrine infusion into Reinke's space (superficial lamina propria [SLP])^{15,17,26,34} improves the preexcisional assessment of lesion depth (Fig 1³⁵) and assists the surgeon's technical execution of the procedure in a number of ways. First, the infusion facilitates mucosal incisions by improving visualization of the lateral border of the lesion and by distending the SLP so that the overlying epithelium is under tension. Second, the infusion also increases the depth of the SLP, thereby facilitating less traumatic dissection in this layer and leading to regenerated epithelium that is more flexible. Third, the epinephrine and hydrostatic pressure of the infusion vasoconstrict the microvasculature in the SLP, thereby improving visualization and precise dissection.

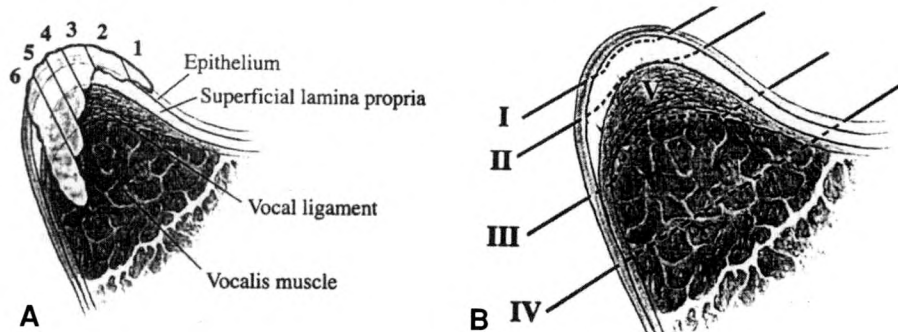


Fig 2. **A)** Diagram displays how surface vocal fold lesion may harbor variety of invasion patterns. **B)** Coronal section of vocal fold delineates depth of potential resection margins for vocal fold atypia and cancer. I — Dissection is performed just underneath basement membrane with preservation of most of underlying superficial lamina propria (SLP); II — dissection is performed well within SLP, but does not include vocal ligament; III — dissection is performed between vocal ligament and vocalis muscle; IV — dissection is performed deep within vocalis muscle. (Reprinted with permission.³⁵)

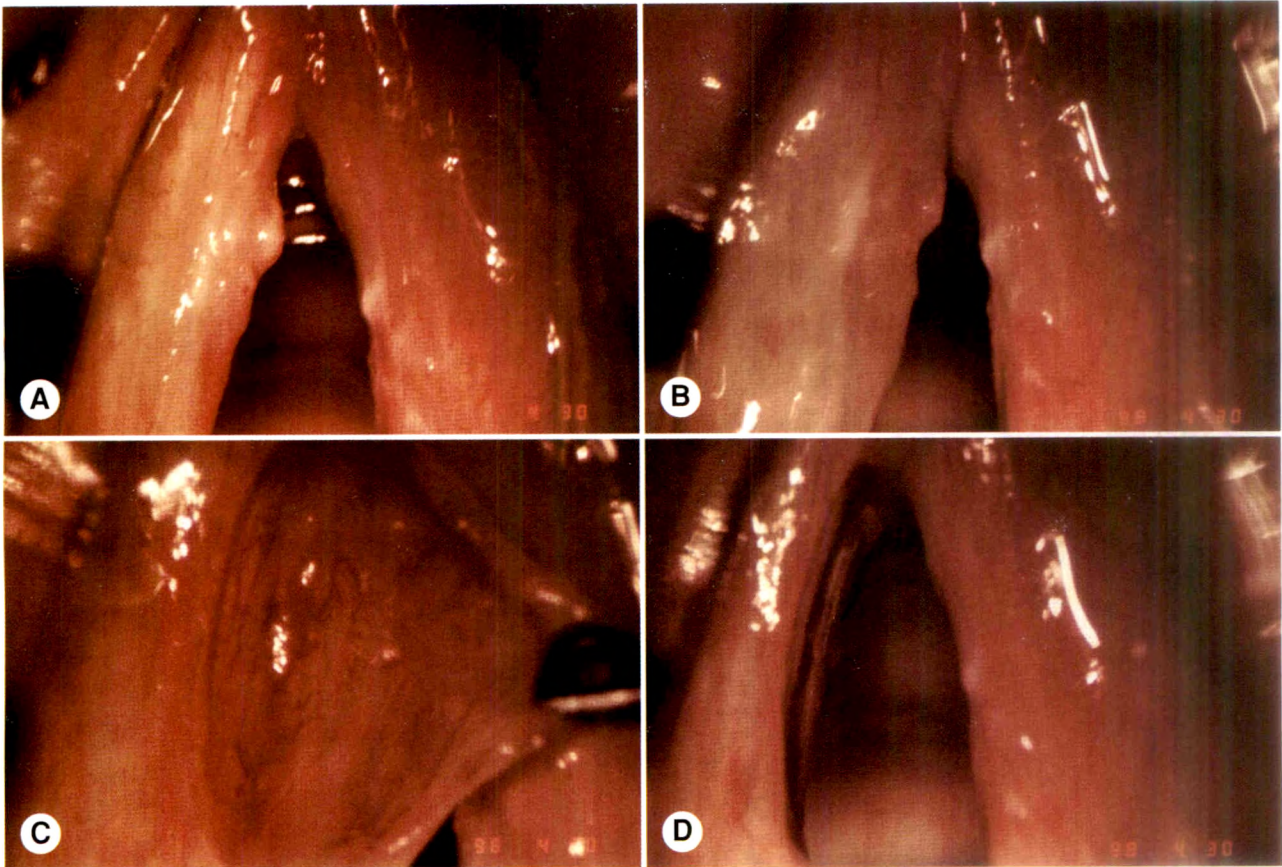


Fig 3. Original $\times 7$. **A)** Bilateral irregular lesions of superior and medial surfaces of vocal folds. There is T1N0M0 squamous cell carcinoma on left and keratosis with atypia on right. **B)** Subepithelial infusion of saline solution with 1/10,000 epinephrine has been done. **C)** Dissection within normal SLP is performed to encompass entire right lesion. Dissection on medial and subcordal surfaces has not begun. **D)** Left-sided lesion has been completely resected. Because of extension to anterior glottal commissure, right-sided lesion will require resection at second stage. (Reprinted with permission.³⁴)

Finally, if a CO₂ laser is used, the saline solution acts as a heat sink, decreasing thermal trauma to the normal vocal fold tissue.

There are 4 basic procedures,¹⁸ which are based on the depth of excision (Fig 2³⁵): 1) dissection just deep to the epithelial basement membrane in the superficial aspect of the SLP, for atypical epithelium and microinvasive cancer (Fig 3³⁴); 2) dissection within the deep aspect of the SLP, for microinvasive cancer that is more extensive but not attached to the vocal ligament (Fig 4); 3) dissection between the deep lamina propria (vocal ligament) and the vocalis muscle, for lesions that are attached to the ligament but not through it; and 4) dissection within the thyroarytenoid (paraglottic) musculature, for lesions penetrating the vocal ligament and invading the vocalis muscle (Fig 5³⁴). This algorithm can be adjusted and fine-tuned further by performing partial resections of any of the layered microstructure. The specimen is always oriented for whole-mount histologic analysis, and frozen-section margin assessment is employed selectively to verify a complete excision.^{17,18}

If the dissection is performed within the SLP, cold

instruments (Fig 4C) facilitate precise tangential dissection around the curving vocal fold. This type of dissection cannot be done with the CO₂ laser without causing unnecessary thermal trauma to the residual SLP.^{17,19} Cold instruments facilitate maximal preservation of the SLP, which allows for optimal pliability of the regenerating epithelium.^{17,19} Dissection between the vocal ligament and the vocalis muscle (Fig 5C) can be performed equally well with cold instruments alone or with the assistance of the CO₂ laser. Dissection within the muscle is performed most precisely with the CO₂ laser (Fig 6B³⁴), which allows for improved visualization because of its hemostatic cutting properties.

Vocal Function Measures. A prospective vocal outcome assessment was done on 32 patients who underwent phonosurgical treatment of early glottic cancer. Preoperative evaluations were obtained in most cases, but were of little value, since a variety of lesion characteristics (ie, endophytic versus exophytic, location on the vocal fold, etc) affect vocal function but do not reflect oncological aggressiveness. The 10 patients who had secondary phonosurgical recon-

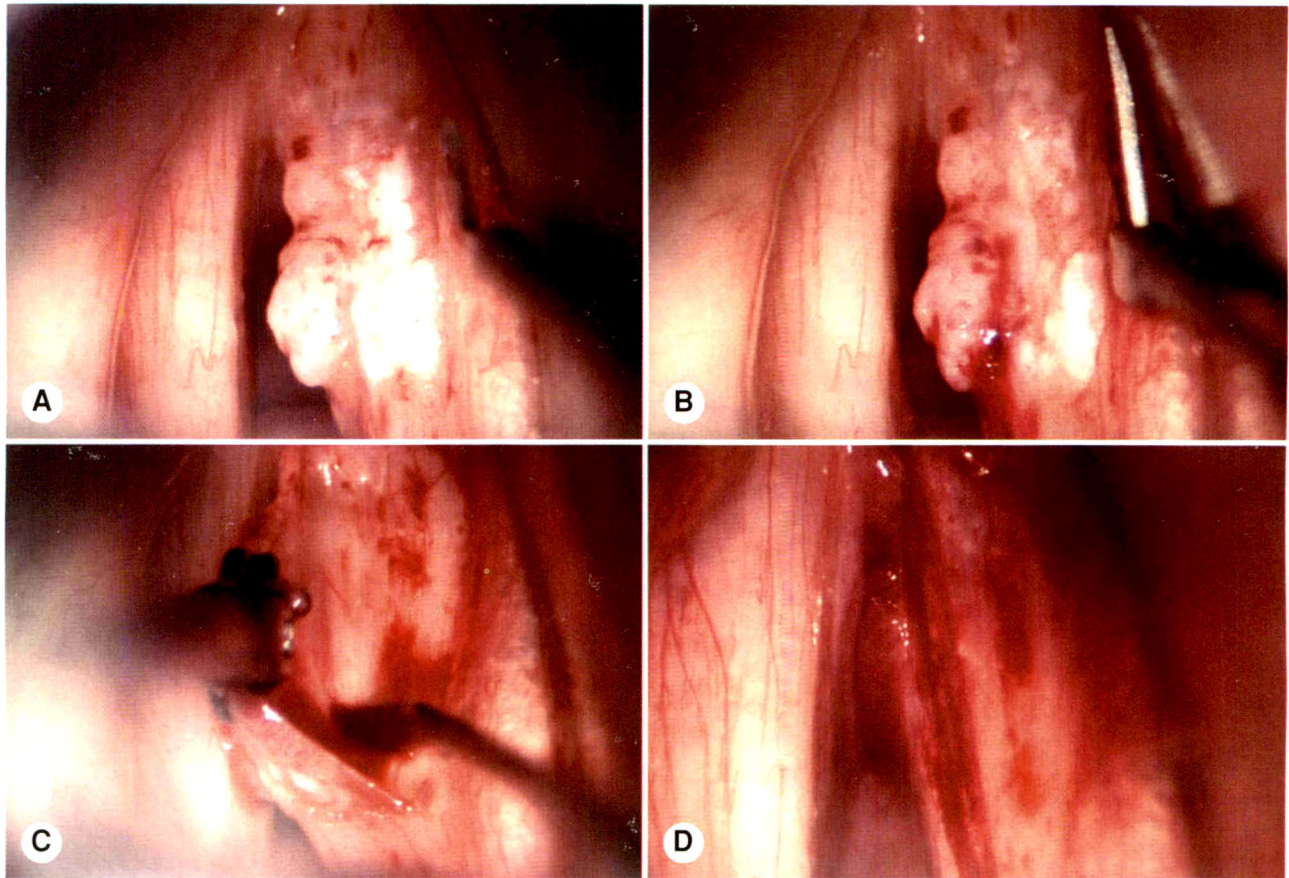


Fig 4. Original $\times 7$. **A)** Professional orator had progressive hoarseness and keratotic lesion of right vocal fold. Infusion needle is seen. **B)** Upturned scissors is used to perform epithelial cordotomy, which serves as 1-mm perimeter margin. **C)** Left curved dissector is used to easily dissect lesion from residual normal SLP. **D)** Resection has been completed with maximal preservation of normal SLP and normal epithelium. Note that there is still thin layer of remaining SLP overlying vocal ligament. (Courtesy of Endocraft LLC.)

struction were also assessed during the time period between the initial surgical resection of their glottic cancer and the subsequent reconstruction, so that the impact of the reconstruction on vocal function could be evaluated. The voice evaluation consisted of videoendoscopy with stroboscopy, as well as acoustic and aerodynamic measurements. The details of this protocol have previously been described.³⁶ Because of the subjectivity of interpretive stroboscopy and its associated shortcomings as an objective measure of vocal function,³⁷ data based on stroboscopic observations are not included in this study. Subjective observations based on stroboscopic examinations are referred to in the Discussion.

Data from the final assessments were compared descriptively to historical norms to examine the extent to which patients' postoperative voice measures approximated normal function. Preconstruction and postreconstruction assessment measures for the 10 patients with reconstruction were compared by paired *t*-tests ($p \leq .01$) to evaluate whether reconstruction had a significant impact on vocal function.

After the final operative procedure for each pa-

tient, a determination was made about the need for voice therapy. Ten of the 32 patients received short-term therapy (average, 2 or 3 sessions) to reduce unnecessary persistent or residual vocal hyperfunction and to enhance vocal fold entrainment (eg, reduce or eliminate diplophonia, etc).

RESULTS

Surgical and Oncological Data. Thirty-two patients (28 with T1a cancer and 4 with T2a cancer) underwent phonomicrosurgical excision of unilateral early glottic cancer. The mean follow-up was 66 months, and all of the 32 patients have had more than 2 years of follow-up. No patient had invasion of the cricoarytenoid joint. There was 1 microinvasive recurrence with the ultranarrow-margin microsurgical approach.¹⁸ That patient underwent a second endoscopic resection without vocal deterioration and has been free of cancer for 37 months.

All patients underwent subepithelial infusion, which facilitated 1) assessment of the depth of invasion and the volume of the lesion and 2) a microflap resection superficial to the vocal ligament within the

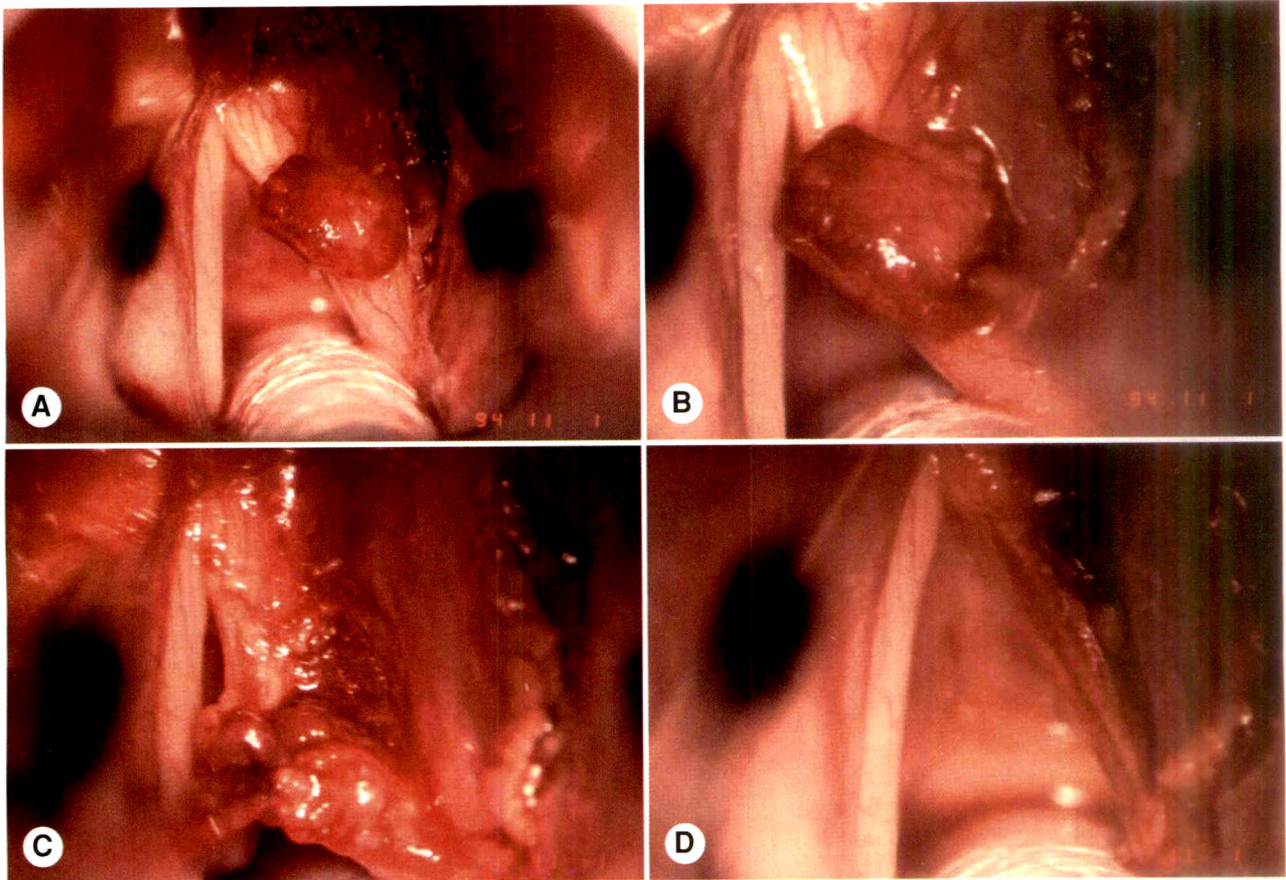


Fig 5. A) Vestibular fold has been resected to provide excellent exposure of right vocal fold cancer (original $\times 4$). B) Prior lesion is seen at higher magnification (original $\times 7$). C) Lesion was attached to vocal ligament, and therefore, resection proceeds with cold instrument dissection between vocal ligament and vocalis muscle (original $\times 7$). Lesion is retracted medially and caudally through glottal aperture. D) Right vocal fold cancer has been resected en bloc so that very little of vocal muscle is disturbed and vocal ligament is used as margin (original $\times 7$). Right neocord is remarkably well contoured, considering extent of resection. (Reprinted with permission.³⁴)

SLP in 15 of the 32 patients. Seventeen lesions required a resection margin deep to the vocal ligament. Nine of the 17 underwent paraglottic muscle resection as well, and all 9 had phonosurgical reconstruction. Of the 8 who underwent resection just underneath the vocal ligament, 1 underwent phonosurgical reconstruction (see Table).

Twenty-two of the 32 lesions required resection of the epithelium overlying the anterior commissure tendon, and in a majority, the tendon was included. Fifteen of the 32 required resection of the epithelium overlying the arytenoid vocal process, and in a majority, the cartilage was included. Anterior and/or posterior surface involvement did not influence local control, as no patient underwent an open laryngectomy or radiotherapy. Twelve of the 32 patients underwent laser resection of a portion of the supraglottis to adequately expose the cancer before its excision.

Vocal Function Measures. The results for all acoustic and aerodynamic measures from the final postop-

erative assessments are displayed in Figs 7-10. Individual patients are coded according to depth of the initial surgical resection: A, SLP; B, vocal ligament; and C, vocalis muscle. In addition, prereconstruction, as well as postreconstruction, measures for the subset of 10 subjects who underwent secondary surgical reconstruction subsequent to the initial cancer resection are also shown.

The results of acoustic assessment of average fundamental frequency (F0) and sound pressure level (SPL) for oral reading are shown in Fig 7 for men and women separately. At the final assessment, 19 of the 26 men (73%) and 4 of the 6 women (67%) displayed F0 values that fell within normal limits (72% overall), and 15 of the men (58%) and 4 of the women (67%) had normal SPL values (59% overall). Four of the 8 men with secondary reconstruction displayed an abnormally high F0 before reconstruction, with 3 of the 4 attaining a normal value after reconstruction. Six of the 8 men (75%) and both women with secondary reconstruction (100%) had reduced SPL values before reconstruction. Five of

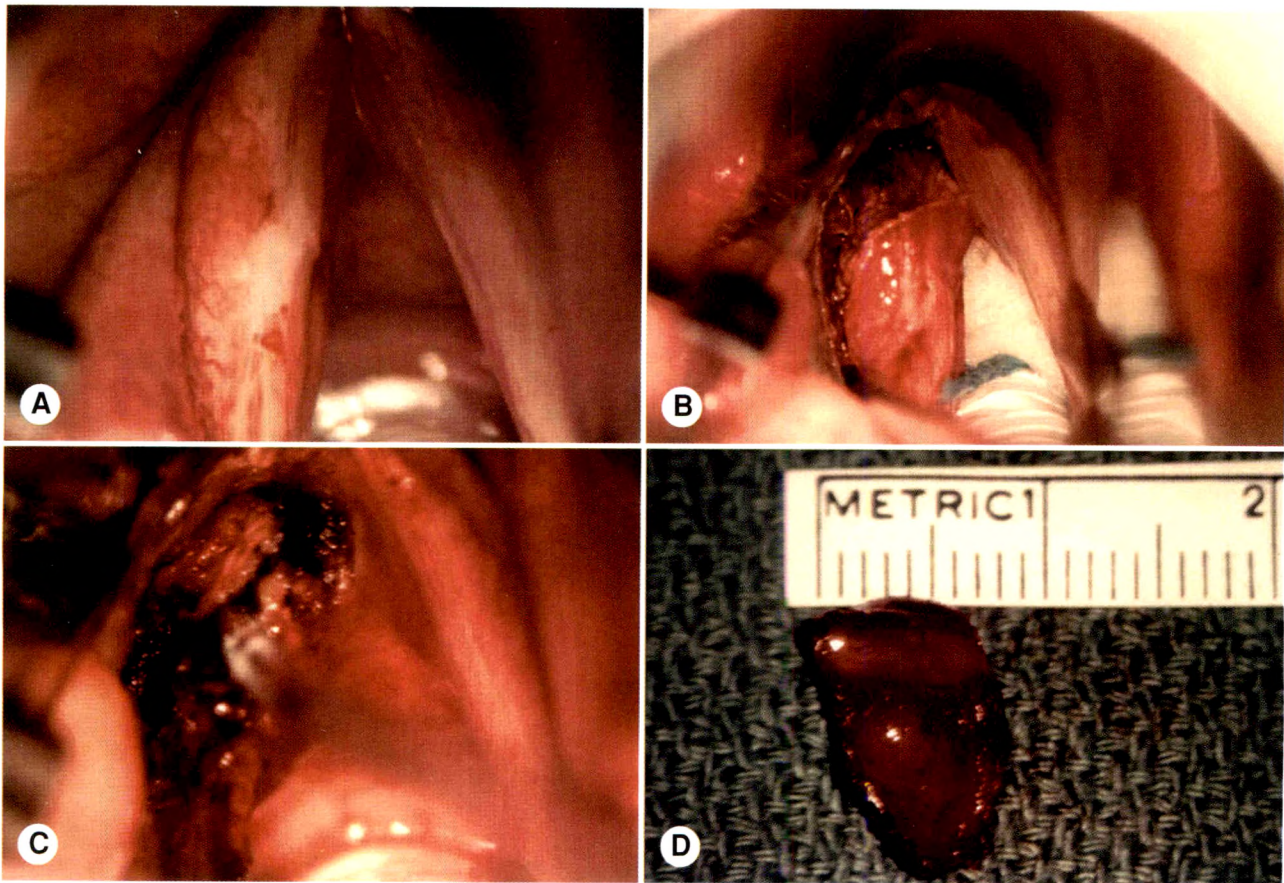


Fig 6. A) Patient presented with previous biopsy (original $\times 7$). Pathology revealed spindle cell carcinoma or carcinosarcoma. There was no substantive mucosal wave. Full length of that vocal fold was uncharacteristically rigid to palpation during laryngoscopy. B) Perimeter incision around thyroarytenoid muscle and its overlying mucosa is made with microspot CO₂ laser (original $\times 4$). C) Thyroid cartilage is seen at edge of carbonization on left (original $\times 7$). Biopsy is done to ensure clear deep margins. D) Resected specimen had submucosal disease. (Reprinted with permission.³⁴)

these 6 men and both women showed postreconstruction increases in SPL, with 3 of the men and both women attaining normal values. The postreconstruction increase in SPL for the entire group was significant at the $p = .006$ level (t -test; 9 df).

The results for voice quality–related measures of F0 perturbation (jitter), amplitude perturbation (shimmer), and noise-to-harmonics ratio (NHR) for sustained vowels are shown in Fig 8 for the entire group of 32 patients. At the final assessment, 14 patients (44%) displayed normal jitter values, 7 (22%) had normal shimmer values, and 24 (75%) had NHR values that fell within normal limits. All 10 reconstructed

patients displayed abnormal values for jitter and shimmer, and 7 had elevated NHR measures before reconstruction. (Note: prereconstruction phonation for subject C6 was too aperiodic to measure.) All of these patients showed reductions toward the normal ranges for all 3 measures after reconstruction, with 3 attaining normal jitter levels, 1 displaying normal shimmer, and 5 having normal NHR values. Subject C6 displayed phonation that was periodic enough to assess after reconstruction, with slightly elevated levels for all 3 measures. The postreconstruction reductions in all 3 measures were significant at the $p \leq .01$ level (t -test; 9 df).

The results for the maximum-range tasks are shown in Fig 9. At the final assessment, 13 of the 26 men (50%) and 5 of the 6 women (83%) had normal maximum F0 range capability (56% overall). In the reconstructed group, 5 of the 8 men and 1 of the 2 women had reduced F0 ranges before reconstruction. Eight of the 10 reconstructed patients (80%) showed increases in F0 range after reconstruction, with 6 of the 10 (60%) attaining normal values. The postreconstruction increase for the group was significant at

PHONOSURGICAL RECONSTRUCTION METHOD

Depth of Resection	No. of Patients	No. With Lipoinjection	No. With Medialization Laryngoplasty
Superficial lamina propria	15	0	0
Ligament	8	0	1
Muscle	9	7	5
Total	32	7	6

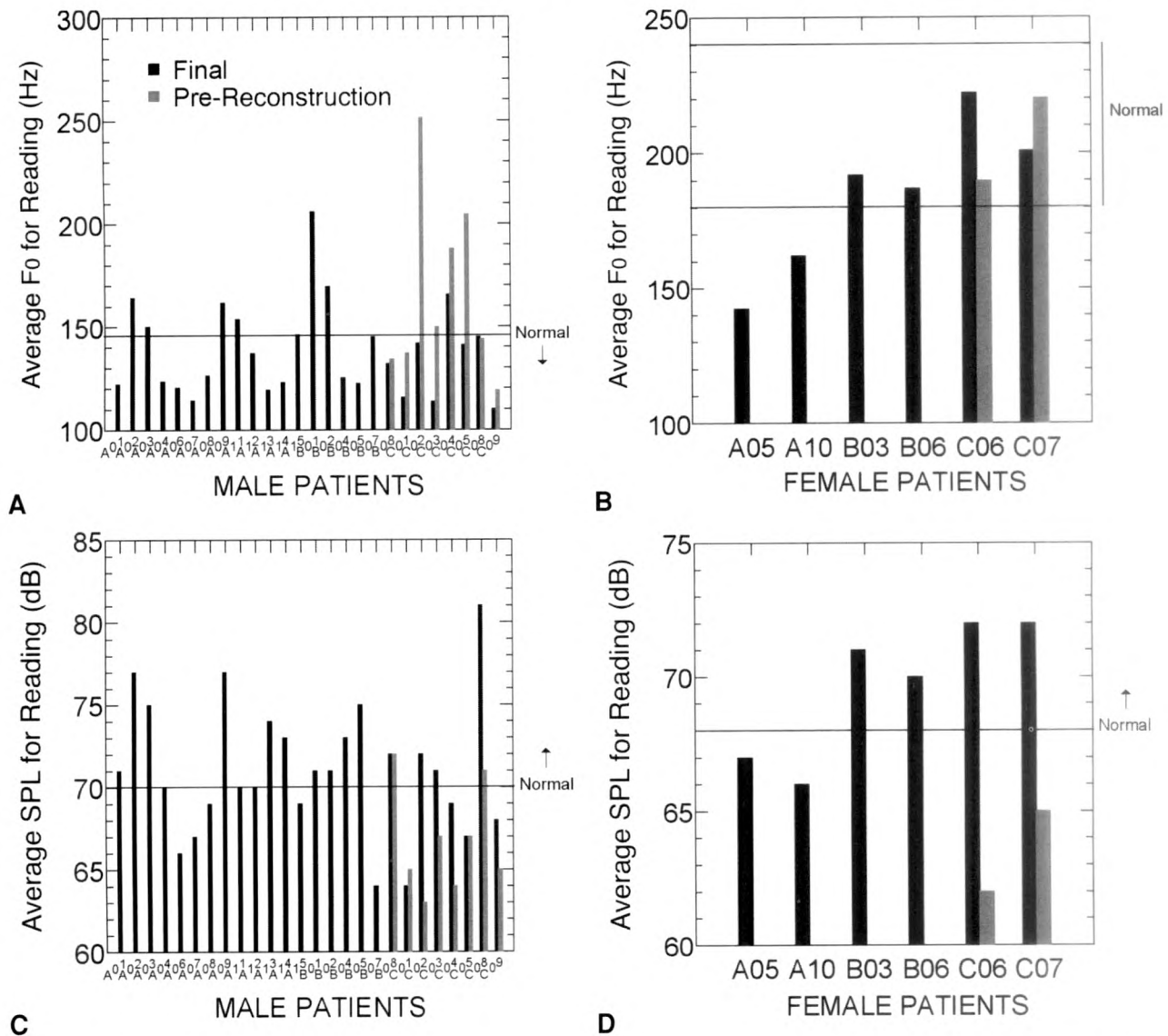


Fig 7. Results for acoustic measures of average **A,B**) fundamental frequency (F0) and **C,D**) sound pressure level (SPL) for reading. Patient codes: A — SLP resection; B — ligament resection; C — muscle resection.

the $p = .008$ level (t -test; 9 df). Nineteen of the 32 patients (59%) displayed normal maximum SPL range values at the final assessment. For the reconstructed group, 8 of the 10 (80%) had reduced SPL range values before reconstruction, with all 10 patients showing postreconstruction increases, and 7 (70%) reaching the normal threshold. The postreconstruction increase in maximum SPL range for the group was significant at the $p \leq .000$ level (t -test; 9 df). Finally, 8 of 23 men (40%; data missing for 3 men) and 2 of the 6 women (33%) displayed normal maximum phonation times at the final assessment. In the reconstructed group, 8 of the 10 (80%) had an increase in phonation time after reconstruction, with 5 (50%) displaying normal values. The postreconstruction increase in phonation time for the group was not statistically significant ($p = .026$; t -test; 9 df).

The results for the aerodynamic measures are shown in Fig 10. Twenty-three of the 26 men (88%) and all 6 women (100%) displayed normal average airflow rates at the final assessment (91% overall). In the reconstructed group, 3 of the 8 men (38%) and both women (100%) had increased airflow rates before reconstruction, with all 5 showing reductions in airflow, and 4 attaining normal values after reconstruction. The reduction in airflow rates for the group was not significant ($p = .132$; t -test; 9 df). Given the strong relationship between subglottal pressure and SPL, it is more meaningful to assess subglottal pressure relative to SPL.³⁸ Thus, subglottal pressure values are plotted versus SPL for individual patients at the final assessment (Fig 10C) and for the pre-reconstruction and postreconstruction assessments (Fig 10D). Also shown on each graph is a line (positive

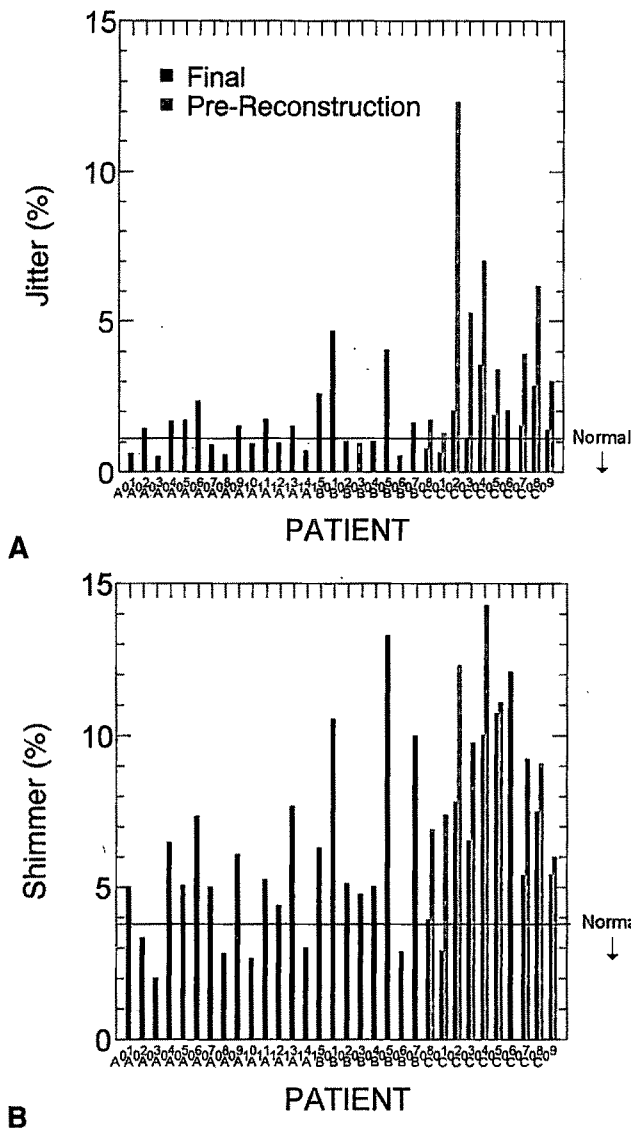


Fig 8. Results for acoustic measures of A) jitter, B) shimmer, and C) noise-to-harmonics ratio for entire group.

slope) representing the average normal relationship between subglottal pressure and SPL. At the final assessment, 28 of the 32 patients (88%) displayed data values that were clearly above the normal line, indicating a tendency to use higher than normal subglottal pressures to attain the measured SPL values. As compared to the preconstruction measures, the postreconstruction values for 8 of the 10 patients showed migration toward the normal line, indicating a reduction in the subglottal pressures used to produce given SPL outputs, ie, an increase in vocal efficiency. The decrease in subglottal pressure ($p = .004$; t -test; 9 df) and the increase in SPL-subglottal pressure ratio ($p = .006$; t -test; 9 df) were both shown to be statistically significant.

DISCUSSION

Philosophy of Management: Disease Assessment and Phonomicrosurgical Resection. A primary parameter by which the clinician should judge the suc-

cess of the treatment of early glottic cancer is the vocal outcome, since any adequate treatment (endoscopic or transcervical excision, radiotherapy) results in a cure rate of around 85% to 90%.³⁹ Recently, laryngologists have incorporated physiological principles of laryngeal sound production into the design of the surgical oncological management (both resection and reconstruction).

A key goal of the endoscopic management of T1 glottic cancer is to narrow the cancer-free margin to minimize patient morbidity while not altering the cure rate. Alternatively stated, the disease must be eradicated with maximal preservation of the vocal folds' normal layered microstructure. In this study, employment of ultranarrow margins resulted in an acceptable 3% rate of local recurrence. Furthermore, no patient required an open resection or irradiation to control the disease. The magnification provided by the surgical microscope is used routinely to provide a 1-mm surface margin (Fig 1B) at the perimeter of the

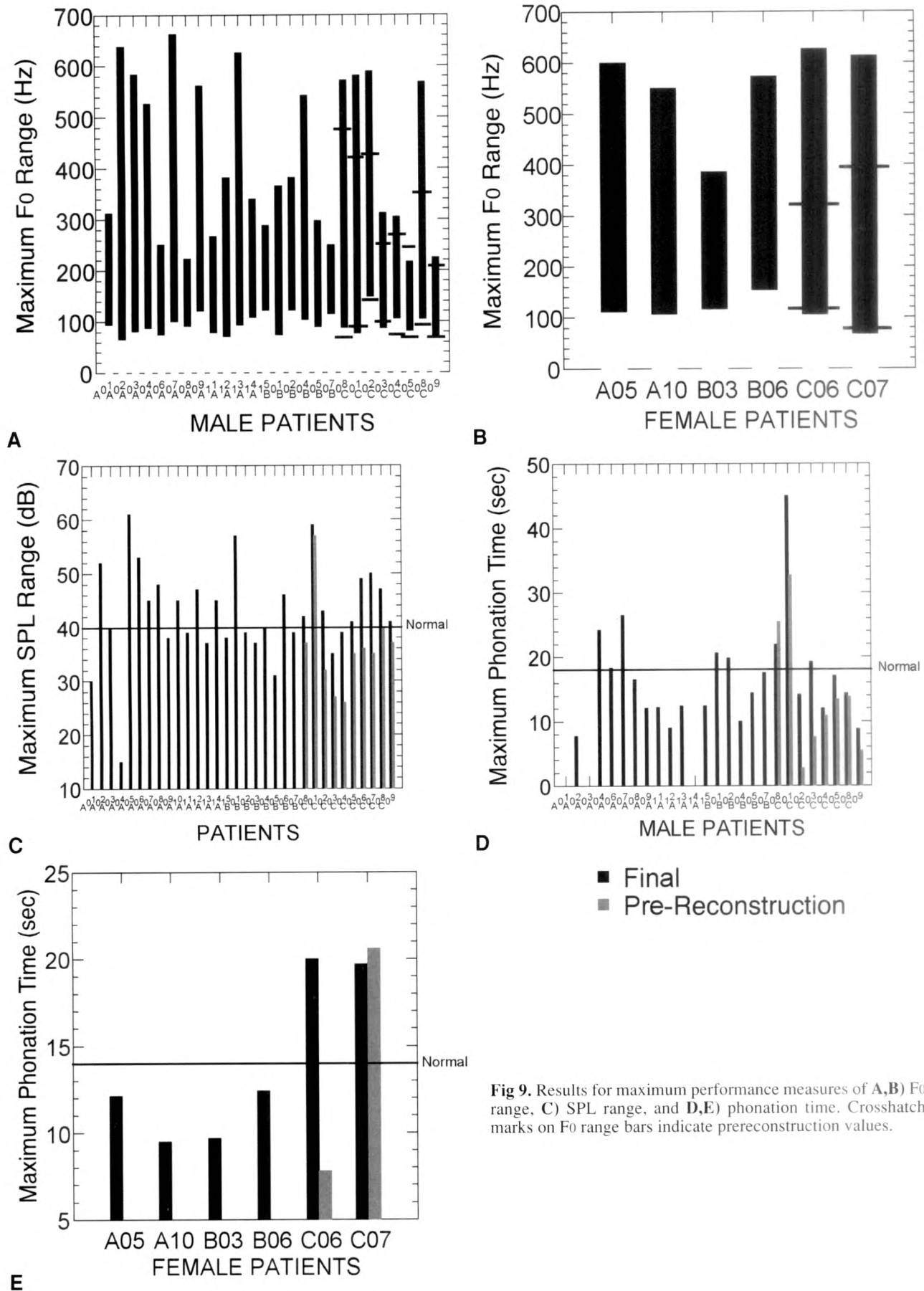


Fig 9. Results for maximum performance measures of A,B) F0 range, C) SPL range, and D,E) phonation time. Crosshatch marks on F0 range bars indicate pre-reconstruction values.

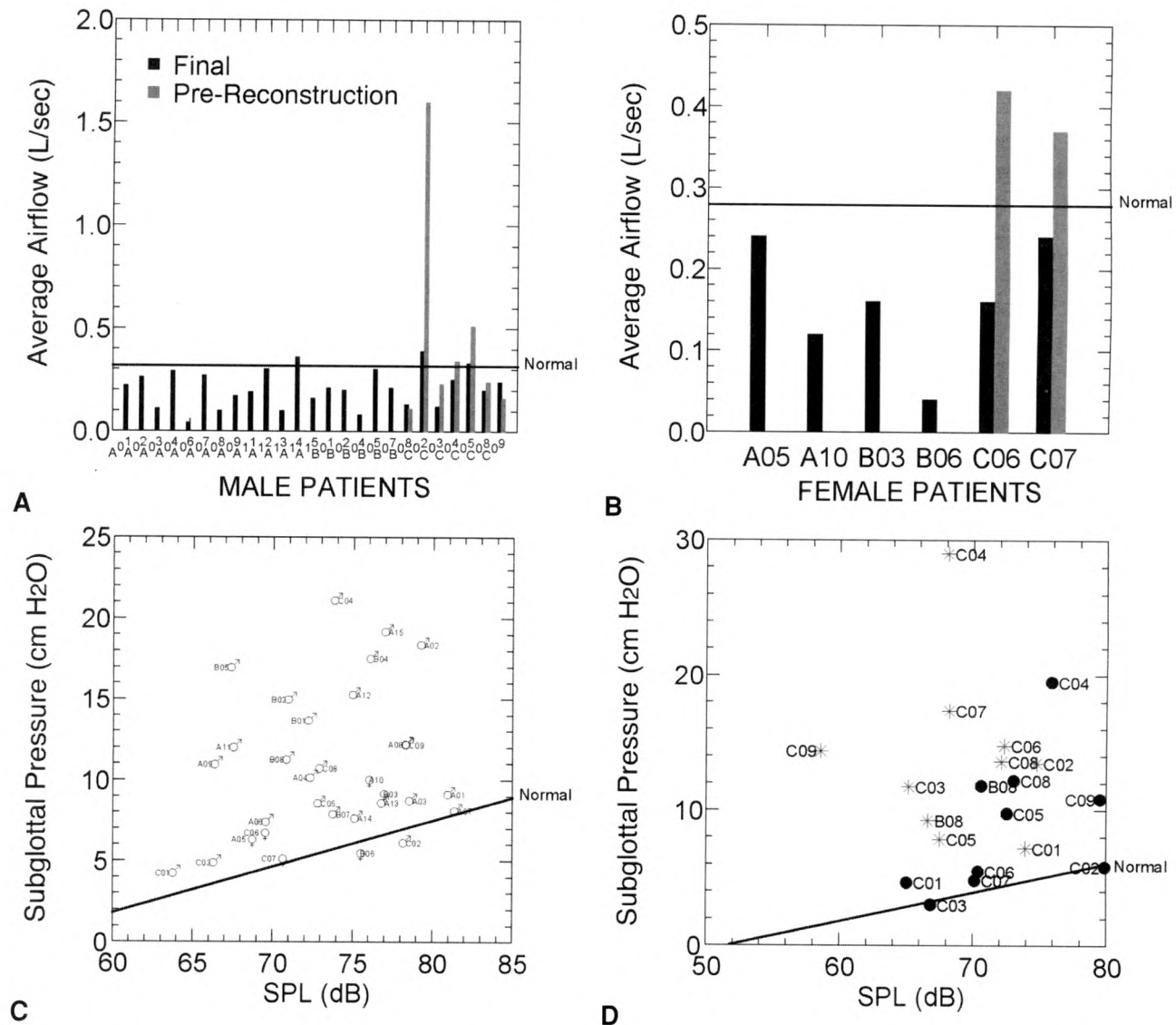


Fig 10. Results for aerodynamic measures of **A,B** average glottal airflow and **C,D** SPL–subglottal pressure ratio. In **D**, asterisks indicate prereconstruction values and solid dots indicate postreconstruction values.

lesion. In the phonomicrosurgical model, a 1- to 2-mm-deep margin is used as well. These procedures precisely accommodate a narrow, deep resection margin based on the depth of invasion (invisible third dimension).

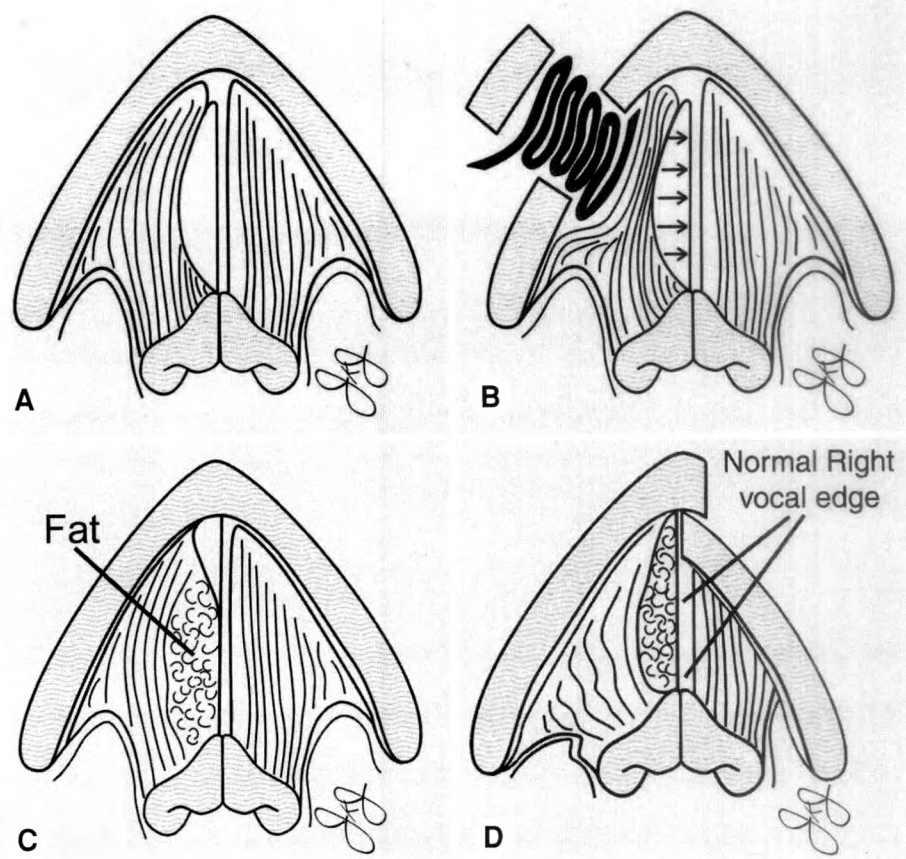
One of the most important features of the subepithelial infusion technique^{15,17} is that it has been instrumental in identifying which lesions are still confined to the SLP (Figs 1A, 3A-C, and 4A-C), the rate of which was surprisingly around 50% in this study. This meant that these lesions could be effectively resected superficial to the vocal ligament with minimal vocal disturbance subsequent to healing and epithelialization. In this investigation, none of the 15 patients who had an SLP resection underwent phonosurgical reconstruction. This is consistent with the findings of Lu et al²² and Delsupehe et al,⁴⁰ who reported that the perceptual vocal outcome was excel-

lent in those patients who could undergo an SLP resection.

A small amount of extra tissue that is excised with the deep margin can have a profound effect on the vocal outcome, while not necessarily improving the cure rate. This is especially so for those lesions that minimally invade the SLP. It was quite remarkable that despite the aggressive surface appearance (Fig 4A) of many lesions with poor mucosal wave propagation,³⁷ almost 50% could be resected superficial to the vocal ligament. As has been previously demonstrated in keratosis with atypia,¹⁷ the magnified appearance of a lesion's surface does not necessarily reflect the severity of the disease.

In a blinded study, it was recently demonstrated that stroboscopy was not reliable in distinguishing keratosis with atypia from cancer, and was only mar-

Fig 11. A) Healed defect subsequent to resection of left vocal fold cancer, which was invading vocalis muscle. B) Gore-Tex medialization of excavated vocal fold edge. C) Lipoinjection method of medialization. D) Subluxation of thyroid laminae. (Reprinted with permission.²¹)



ginally helpful in determining the depth of cancer invasion.³⁷ The most valuable asset of stroboscopy in the evaluation of dysplasia and cancer of the glottis is the determination of the pliability of vocal fold epithelium that is without disease, since this soft tissue will be primarily responsible for posttreatment vocal fold vibration and the resultant vocal quality. Careful assessment of the functioning SLP underlying the uninvolved epithelium allows the clinician to prognosticate the vocal outcome from the cancer treatment. It is not unusual for patients with T1a glottic cancer to have abnormal contralateral mucosal waves as a result of long-term phonotrauma related to their personality or vocation. Furthermore, phonation with a neoplastic lesion typically leads to hyperfunction, high subglottal pressures, and increased collision trauma. Most likely, patients with early glottic cancer have had prior atypia and therefore have probably had a glottal lesion for years, not to mention the other long-term negative effects of smoking and other environmental abuses on the vocal folds.

Given the various factors that can contribute to degraded vocal function in this patient cohort, the use of available normative data for comparison purposes clearly creates a bias toward finding abnormal results for the glottic cancer group, regardless of treatment modality. The use of control data from a group

of subjects matched not only for age, but also for smoking history, vocation, voice use profile, etc, would probably enable better isolation of the effects of the cancer and its treatment on vocal function. We have previously described a similar control group issue with respect to evaluating vocal outcomes in patients undergoing surgical treatment for Reinke's edema.⁴¹

Phonomicrosurgical Postresection Vocal Outcome. Regardless of treatment modality, there is subsequent unavoidable stiffness of previously cancerous vocal fold epithelium, and the dominant oscillator becomes the contralateral SLP and overlying epithelium. The laryngologist can precisely accommodate a microcontrolled excision to the 3-dimensional characteristics of the vocal fold cancer. Therefore, the graded resection approach that was suggested by Hirano et al⁴² and Koufman⁴³ was developed further.^{17,18} If glottal closure and aerodynamic efficiency are maintained, the plasticity of the vocal system (ie, increasing subglottal pressure) can usually compensate to achieve a normal or near-normal conversational-level voice. Unlike irradiation,⁴⁴ phonomicrosurgery avoids trauma to the layered microstructure of the uninvolved contralateral fold, which is ultimately the primary glottal sound source. Furthermore, the mucosal saccular glands are less affected by surgery.

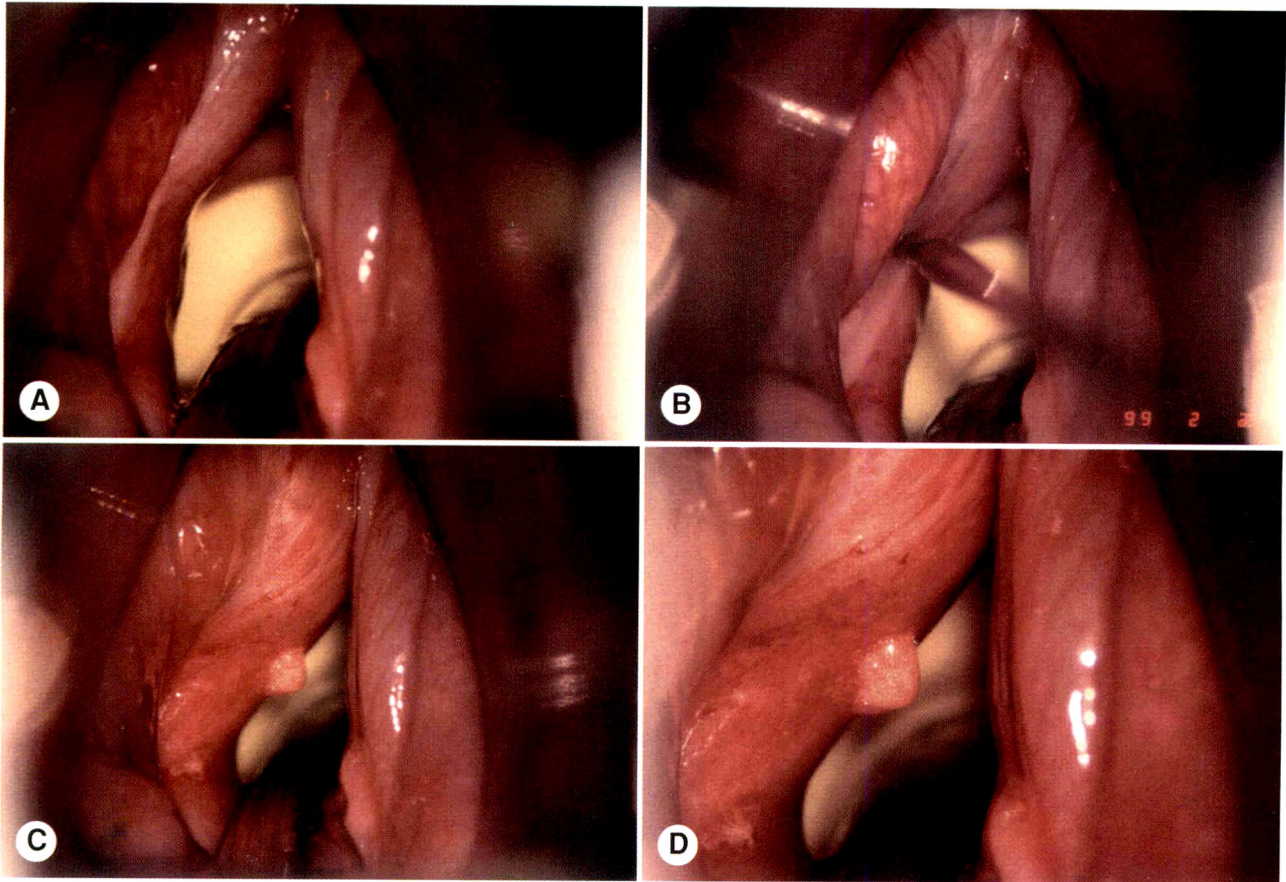


Fig 12. A) Patient has undergone phonosurgical resection of left T1a carcinoma and now has excavated vocal fold in musculomembranous region (original $\times 4$). B) Brunings injection needle is placed lateral to concavity in paraglottic space (original $\times 4$). C) Vocal fold has been augmented with fat (original $\times 4$). Small amount of graft is extruding from puncture site and should be removed with cold instruments to avoid granuloma. Note new convexity of vocal fold. D) Image in C at higher magnification (original $\times 7$). (Reprinted with permission.²⁰)

When the surgical dissection is confined to the SLP in one vocal fold, the perceptual impression of the resulting voice usually approximates normal if the contralateral vocal fold is normal. Stroboscopy often reveals that there is some mucosal wave propagation as a result of preserving some residual SLP. We have not seen evidence that the pliable SLP regenerates after removal in the way that epithelium

does. Epithelium assumes the viscoelastic properties of the tissues underlying it, which is the soft tissue that is preserved. If the cancer margin is confined to the SLP, most patients can comfortably increase subglottal driving pressures to achieve function that approximates normal voice at conversational levels, both in terms of subjective perception and objective measures. For example, a clear majority of the “SLP”

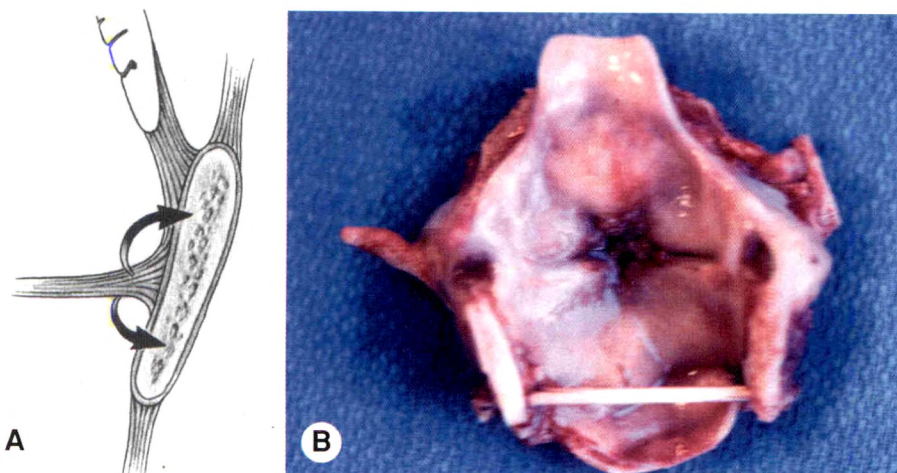


Fig 13. A) Diagrammatic representation of how cancer spreads around anterior commissure tendon to invade thyroid cartilage. (Reprinted with permission.³⁵) B) Old laryngectomy specimen. Patient failed radiotherapy and had severe pulmonary compromise. Diffuse soft tissue invasion cephalad and caudal to anterior commissure tendon correctly portended thyroid cartilage invasion. (Reprinted with permission.³⁴)

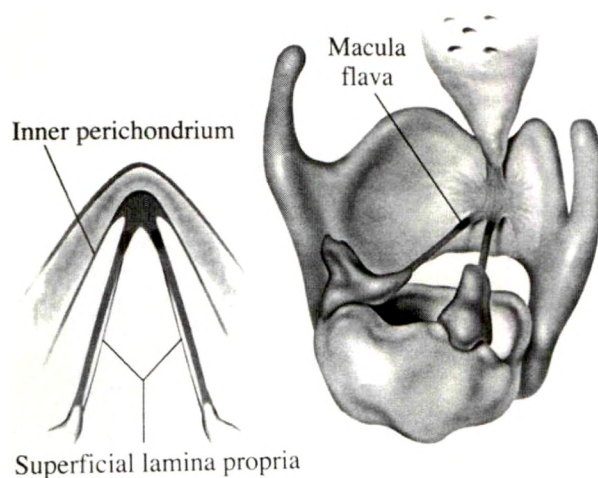


Fig 14. Diagrammatic representation of anterior commissure tendon. (Reprinted with permission.³⁵)

patients in this study had normal values for average F0, NHR, and average glottal airflow. Fewer of the SLP patients attained normal levels of function when attempting maximum performance tasks (phonation time, F0, and SPL ranges) outside of the conversational range. For the additional voice quality–related measures that did not show normal values for a majority of SLP patients (jitter and shimmer), it is important to note that aside from a few exceptions, most of the abnormal values fell relatively close to the normal threshold that was used.

After a resection of the vocal fold epithelium and its underlying laminae propria (SLP and vocal ligament), the healed neocord is usually smooth and variably concave, depending on the magnitude of ligament and/or muscle resection. Those patients who require only the vocal ligament to be resected as a deep margin typically heal with a smooth and straight vocal edge; however, there is unavoidable loss of epithelial pliability. If postsurgical excavation of the vocal edge does not occur, the vocal function resembles that following an SLP dissection. However, in some of these individuals, the medial surface will have a slight concavity that is correctable by means of phonosurgical medialization. This is clearly demonstrated by the vocal function results for the only ligament resection subject (subject B08) that underwent subsequent medialization. This patient showed improvements in almost all of the relevant measures, with values for jitter, shimmer, maximum SPL range, and maximum F0 range going from abnormal to within normal limits.

Given the deeper resection of the “ligament” group, it was surprising that the postresection voice results were comparable to those of the SLP cohort. This outcome suggests that the surgeon should not attempt oncological brinkmanship by performing an SLP dis-



Fig 15. Appearance of neck of individual at conclusion of irradiation for T1 glottic carcinoma. (Reprinted with permission.³⁴)

section. However, there was a slight trend toward improved vocal function, with the SLP group displaying lower average values for voice quality–related measures (jitter, shimmer, NHR) and subglottal pressure, larger average values for maximum performance tasks (SPL and F0 ranges), and larger SPL–subglottal pressure ratios. Larger numbers of patients are needed to formally evaluate whether this trend indicating better vocal function in the SLP group than in the ligament resection group is truly significant. Additionally, retaining the vocal ligament allows for future restoration of the entire layered microstructure of the vocal fold as methods of SLP augmentation are designed.^{45,46}

As the deep resection margin extends to include the paraglottic musculature, there is further danger of aerodynamic and acoustic impairment. Increasing the magnitude of the resection, especially its depth, leads to deteriorating vocal function that may be associated with aerodynamic leakage with unavoidable loss of epithelial pliability.^{17,42,47} These patients are optimal candidates for phonosurgical reconstruction, as demonstrated by the results for the 9 patients that underwent reconstruction medialization subsequent to resection into the vocalis muscle. As a group, these patients displayed notable postreconstruction improvements in measures of average SPL, all 3 voice quality–related measures (jitter, shimmer, NHR), maximum ranges for SPL and F0, subglottal pressure, and SPL–subglottal pressure ratio. In fact, a majority of these patients had attained normal values for many of the vocal function measures by the final assessment. These results are similar to those of the perceptual vocal outcome comparison between CO₂ laser resection and radiotherapy done by McGuirt et al.⁴⁸ In that study, physicians and patients perceptu-

ally rated the irradiated group as slightly better, presumably because their resection model utilized wider, deeper margins and the patients did not have reconstruction.

Phonosurgical Reconstruction and Voice Rehabilitation. Patients who did not require resection of muscle did not typically require phonosurgical reconstruction in the form of an augmentation and/or medialization, although it is possible that their vocal function would have been enhanced by such procedures. Deeper resections often resulted in a healed neocord that was concave. The excavated neocord resulted in incomplete glottal closure and a tendency to generate higher airflows during phonation. The result was a stiff, leaking glottal valve and a hoarse, disordered voice.

Valvular incompetence of the glottis resulted in increased subglottal pressures and flows, increased acoustic instability (jitter and shimmer), and increased supraglottal muscle strain patterns. Subjective stroboscopy reveals no ipsilateral substantive mucosal wave oscillation, but rather, a mass vibration of the stiff neocord body.¹⁷

Once the neocord is healed, phonosurgical reconstruction may be done if the vocal edge is excavated. As necessary, a medialization laryngoplasty was performed if there was adequate glottal tissue medial to the thyroid lamina.^{21,22} Gore-Tex has been ideal as an implant, since it can be molded in vivo (Fig 11B) to conform to the abnormal postresection contours.^{21,49} The advantage of medialization is that it is done under local anesthesia with intravenous sedation, which allows for phonatory feedback during the procedure. As stated above, the vocal outcome is primarily dependent on the flexibility of the normal layered microstructure of the uninvolved vocal fold.

In this study, when a large amount of paraglottic soft tissue was resected, it was advantageous to perform an initial microlaryngoscopic lipoinjection under general anesthesia to augment the paraglottic region. If there was persistent glottal incompetence, this lipoinjection facilitated subsequent implant medialization. Some clinicians generally prefer lipoinjection (Figs 11C and 12²⁰) as a medialization procedure^{21,50} and will use this method as a primary reconstruction of smaller deficits.⁵¹ Subluxation of the anterior thyroid laminae (Fig 11C,D) can also close a keyhole aperture anteriorly if the anterior commissure tendon has been resected.²¹

Patients who were candidates for these larger transoral procedures (endoscopic vertical hemilaryngectomy) were treated by means of an open transcervical hemilaryngectomy during much of the 20th century. Since this was a confined soft tissue disease, Som

indicated that the thyroid lamina be removed lateral to the paraglottic muscle to collapse the glottal valve for an optimal vocal outcome, not for oncological safety. In the current paradigm, a minimally invasive endoscopic resection approach is used to resect the soft tissue, and the cartilage framework is retained to serve as a scaffolding and buttress for the phonosurgical reconstruction.

There has not been significant concern that performing a window laryngoplasty compromises oncological management by removing a barrier (cartilage framework) to the spread of a potential future recurrence. First, recurrences are very rare, and when they do occur, they are easily seen in the glottal introitus. Second, removing the thyroid ala for voice enhancement during transcervical vertical partial hemilaryngectomy has been a gold standard for decades in the management of larger neoplasms.

If the anterior commissure tendon has been resected (see below) unilaterally, the patient will often have an anterior keyhole aperture during adduction, since soft tissue fibrosis does not fill in the deficit and the epithelium regenerates on the underlying thyroid lamina. The persistent aperture must be closed for adequate voice restoration. A procedure was designed recently that comprises an anterior laryngofissure (under local anesthesia) and subluxation of the thyroid alae.²¹ It resulted in dramatic improvement in voice quality and aerodynamic efficiency, as demonstrated by the results for patients C02 and C08. Both of these patients displayed marked improvements on all 3 voice quality-related measures (jitter, shimmer, NHR), on all 3 maximum performance tasks (phonation time, SPL range, F0 range), and in terms of average SPL. Patient C02 also showed dramatic improvements in average airflow, average F0, and subglottal pressure, with an associated increase in the SPL—subglottal pressure ratio (vocal efficiency).

A total of 10 patients received voice therapy after the final postoperative procedure. These patients received an average of 2 to 3 sessions that focused primarily on reducing any persistent vocal hyperfunction and on exercises designed to enhance vocal fold entrainment. We believe that such postoperative voice therapy is crucial to optimizing postsurgical vocal outcomes for a segment of this patient cohort.

Disease at Anterior Commissure and/or Arytenoid Cartilage. In this study, disease at the anterior glottal commissure or the arytenoid cartilage was not a problem to resect^{23,31,34} with specially designed glottoscopes²⁸ that facilitate optimal exposure.²³⁻²⁵ The examining tube of the universal modular glottiscope has a lancet-arch triangular shape to accommodate to the soft tissues of the glottis and the conformation

of the inner contours of the cartilaginous thyroid alae. This shape facilitates maximal exposure of the superior surface of the anterior commissure, as well as the ventricular mucosa and the lateral deep paraglottic musculature.²⁸

With adequate exposure, up to 70% of the anterior commissure tendon can be resected without dissociating the contralateral vocal fold from its anterior attachment to the thyroid lamina; this maneuver has significant postoperative ramifications for vocal quality. As necessary, an endoscopic CO₂ laser exploration and resection of the infrapetiole region of the supraglottis allows for definitive determination about whether a presumed T1 lesion has cartilage invasion (T4; Fig 13^{34,35}). Cephalad exposure of the glottal soft tissues facilitates precise staging (without disarticulating Broyle's ligament) for selection of commensurate management that will optimize posttreatment voice quality.^{33,35}

The anterior commissure tendon (Broyle's ligament)⁵² is a confluence of the vocal ligament, the thyroepiglottic ligament, the conus elasticus, and the internal perichondrium of the thyroid alae (Fig 14³⁵). There is a misconception that T1 cancers at the anterior commissure have a great predilection for understaging and that many of these lesions have occult invasion of the thyroid cartilage (T4 stage).^{53,54} This belief is based upon the misunderstanding that the anatomy of the dense anterior commissure ligament is a less resilient tumor barrier than the adjacent thin thyroid perichondrium. Kirchner et al^{53,54} have made clear that T1a and T1b carcinomas rarely transgress Broyle's ligament to invade thyroid cartilage. Anterior commissure tumors that have thyroid cartilage invasion typically display cephalad surface invasion of the infrapetiole region of the supraglottis or caudal surface invasion of the subglottis (both T2 by surface staging criteria; Fig 13).

There have been divergent opinions as to whether cancer can be endoscopically eradicated from the anterior commissure. The proscriptions imposed by some are based primarily on the difficulty of obtaining adequate surgical exposure in this area.⁵⁵ The rate-limiting factors for resection of early cancer in the anterior commissure are the true extent of the disease (ie, whether it is invading cartilage) and the endoscopic exposure required to encompass the lesion. Davis et al⁵⁶ and the Boston University group and, later, Koufman⁴³ demonstrated that cancer could be removed from the anterior commissure; however, it required great skill to excise the lesion without vaporizing the specimen. The technique has become less difficult with newer laryngoscopes²⁸ designed to solve the exposure problem by conforming to the

inner contours of the thyroid laminae.^{28,35} Vaporizing cancer without clear en bloc resection margins is inadequate surgical oncological technique. This factor, as well as underestimating the extent of disease, probably led to the failures reported by a number of investigators.^{55,57-59}

Because of the treatment failures mentioned above, poor outcome was mistakenly attributed to the biological behavior of the disease, rather than to inadequate exposure and compromised resection.^{55,57-59} In addition to improved laryngoscopes, a recently designed procedure facilitates the diagnostic staging and treatment of early glottic cancer at the anterior commissure.^{33,35} It involves resecting the supraglottal tissue (infrapetiole region and anterior vestibular folds) cephalad to the anterior commissure tendon to determine whether limited anterior glottic cancer has invaded the thyroid cartilage.

A CO₂ laser resection of the uninvolved supraglottis was done in 12 cases in which the perimeter of the lesion could not be visualized in its entirety. This resection allowed for positioning of the triangular glottiscope adjacent to the inner thyroid lamina above the anterior commissure, which facilitated clear resection of part or all of the anterior commissure tendon. If a cancer is confined to the anterior commissure tendon and if resection of the tendon is not in the patient's best interest (ie, for postoperative voice quality), irradiation can be started promptly in the knowledge that there is true T1 disease. The subcordal-subglottic region is best assessed by means of laryngeal telescopes.

If one accepts the histopathologic data of Kirchner et al^{53,54} regarding the invasion pattern of T1 glottic cancer, there is no reason to believe that an adequate soft tissue resection of small-volume soft tissue disease is not adequate treatment. This premise is further substantiated by a number of reports that did not find a correlation between anterior commissure involvement with T1 glottic cancer and failure of radiotherapy as a curative modality.^{60,61}

The extensive European experience reported by Steiner,⁶² Eckel and Thumfart,⁶³ and Rudert⁶⁴ substantiates that glottic cancer in the anterior commissure can be removed transorally. The problem with any surgical approach to the anterior commissure (for true T1a, T1b, and T2 lesions) is that when these procedures disturb the structural integrity of both sides of the anterior commissure tendon, vocal quality is more significantly impaired. Lesions that are invading cartilage are T4 by stage, require open partial laryngectomy, and are not classically suitable for endoscopic excision.

Phonomicrosurgical Resection Versus Radiotherapy. There have been a number of recent studies that have compared the vocal function of endoscopic resection and radiotherapy. It was not surprising that these studies generally suggested that radiotherapy resulted in equivalent or superior voice quality.^{48,65} The surgical arm of these studies did not include more recent developments. There were no subjects who underwent resection with ultranarrow SLP margins^{18,34,40} or who underwent reconstruction subsequent to larger resections. Essentially, some patients probably underwent more extensive resections than were necessary, and others, with larger postresection excavations, did not undergo phonosurgical medialization. In the present study, these 2 groups comprised 24 of the 32 patients. We are currently comparing the vocal outcome of our surgical cohort to that of a matched population who underwent radiotherapy.

There are a number of advantages of endoscopic excision as compared with radiotherapy in the treatment of early glottic cancer. Phonomicrosurgical resection preserves all treatment options, including further transoral resections. Radiotherapy (Fig 15³⁴) unavoidably treats all normal glottal tissue, as well as the saccular glands, which provide moisture that enhances vocal fold vibration. Postradiotherapy videostroboscopic examinations suggest that both normal and cancerous tissues reflect fibrotic changes and impaired mucosal oscillation.⁴⁴ The use of radiotherapy for early cancers precludes its further use for tumor recurrence or for new primaries, and may even induce carcinogenesis in atypical epithelium.^{66,67} The cost of endoscopic excision is significantly less than that of open laryngeal surgery or irradiation, since either of these latter procedures is typically preceded by a staging endoscopy.^{39,68} Also, the radiotherapy patient sustains an increased burden of time and travel commitments.

Comparing the voice results in the present investigation with those from other studies of different treatment modalities for early glottic cancer is somewhat problematic, because there is wide variation across studies in terms of the approaches used for evaluating vocal function outcomes. After setting aside the studies that have exclusively examined laser excision, which are not comparable for the reasons already given, there is a small group left of investigations that examine radiotherapy treatment for early glottic cancer.

An even smaller subset of such studies includes attempts to examine the impact of radiotherapy on voice outcomes by using more than just subjective

data based on listener or patient rating scales. Investigations that have used vocal function measures to track voice changes across radiotherapy treatment have mostly reported improvements in voice starting several months after radiotherapy,^{69,70} but it is not always clear the extent to which the vocal function approximates normal. The investigations that have compared vocal function measures to normal or control data have reported contradictory results, ranging from reports of normal or near-normal voice following radiotherapy for a majority of patients^{65,71} to just the opposite, with most patients displaying abnormal postirradiation voices.^{44,72-74} In general, the results from the present study compare quite favorably with the radiotherapy studies that show normal or near-normal posttreatment measures, particularly with respect to comparable acoustic measures of perturbation, average F0, and ranges for F0 and SPL.^{65,71}

CONCLUSIONS

1. Early glottic cancer can be resected relatively safely and effectively with ultranarrow margins.
2. A conversationally normal or near-normal voice can be achieved in a majority of patients by means of phonosurgical management (resection and reconstruction) in most patients, regardless of the depth of disease, if the contralateral vocal fold is anatomically intact.
3. After phonomicrosurgical resection, restoration of vocal function is based on reestablishing aerodynamic glottal competency by means of a straight neocord.
4. In this study, the subepithelial infusion technique allowed for effective selection of patients who did not require a resection deep to the vocal ligament, regardless of surface appearance.
5. Patients who did not require resection deep to the vocal ligament did not require reconstruction.
6. Most patients who lost paraglottic musculature benefited from medialization and augmentation to reestablish aerodynamic efficiency.
7. In this study, anterior commissure or vocal process involvement did not correlate with local cancer recurrence.
8. Subjective surgical observations revealed that it was easier to control the phonosurgical outcome with transcervical medialization laryngoplasty than with lipoinjection, since there was phonatory feedback from the patient, enhanced intraoperative adjustability and precision, and no postoperative absorption.

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PHONOMICROSURGERY IN SINGERS AND PERFORMING ARTISTS: TREATMENT OUTCOMES, MANAGEMENT THEORIES, AND FUTURE DIRECTIONS

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Phonomicrosurgery in performing artists has historically been approached with great trepidation, and vocal outcome data are sparse. The vocal liability of surgically disturbing the superficial lamina propria (SLP) and epithelium must be balanced with the inherent detrimental vocal effect of the lesion(s). A prospective investigation was performed on 185 performing artists who underwent phonomicrosurgical resection of 365 lesions: 201 nodules, 71 polyps, 66 varices and ectasias, 13 cysts, 8 keratotic lesions, 2 granulomas, 2 Reinke's edema, and 2 papillomas. Nearly all patients with SLP lesions reported improvement in their postsurgical vocal function. This subjective result was supported by objective acoustic and aerodynamic measures. All postsurgical objective vocal function measures fell within normal limits, including a few that displayed presurgical abnormalities. However, given the relative insensitivity of standard objective measures to assess higher-level vocal performance-related factors, it is even more noteworthy that 8 of 24 objective measures displayed statistically significant postsurgical improvements in vocal function. Such changes in objective measures mostly reflect overall enhancement in the efficiency of voice production. Phonomicrosurgical resection of vocal fold lesions in performing artists is enjoying an expanding role because of a variety of improvements in diagnostic assessment, surgical instrumentation and techniques, and specialized rehabilitation. Most of these lesions are the result of phonotrauma and arise within the SLP. Successful management depends on prudent patient selection and counseling, ultraprecise technique, and vigorous vocal rehabilitation. Furthermore, an understanding of the vocal function and dysfunction of this high-performance population provides all otolaryngologists who manage laryngeal problems with valuable information that they can extrapolate for use in their practices.

KEY WORDS — dysphonia, ectasia, laryngoscopy, microlaryngoscopy, microsurgery, nodule, phonomicrosurgery, phonosurgery, polyp, singer, varix, vocal cord, vocal fold, voice.

INTRODUCTION

Endolaryngeal management and resection of lesions on the vocal fold edge in singers and performing artists¹⁻⁵ has been done since the 19th century, when mirror-guided removal of lesions was introduced.⁶ The critical pathway for decision-making that faced the patient and surgeon then remains unchanged today. Cooperatively, the patient and surgeon must carefully assess whether the lesion(s) are of greater liability to the vocal instrument and career than is the operation to excise the lesion(s).⁷ This judgment requires detailed patient-surgeon communication and *mutual responsibility* for the chosen management course.

In 1895, Kirstein⁸ correctly predicted that direct laryngoscopy would supplant indirect laryngoscopy as the preferred technique for endolaryngeal surgery. Furthermore, every substantive 20th-century addition

to Kirstein's method⁹ improved its precision, eg, the stability of the operating field provided by general endotracheal anesthesia with paralysis.¹⁰ Despite the fact that Kirstein predicted the value of laryngeal stroboscopy (see also Oertel¹¹ from 1895) for enhancing direct laryngoscopy, it was not until the end of the 20th century that stroboscopy was used routinely in association with vocal fold surgery. During the past decade, the convergence of physiological theories of mucosal wave oscillation¹²⁻¹⁵ with improvements in microlaryngeal instrumentation and techniques¹⁶⁻²⁵ has led to current initiatives in phonomicrosurgery.^{7,22,23,26-28} The premise underlying successful phonomicrosurgery is maximal preservation of the vocal folds' layered microstructure (epithelium and laminae propria) in order to optimize glottal closure and epithelial pliability.

The primary goal of this investigation was to as-

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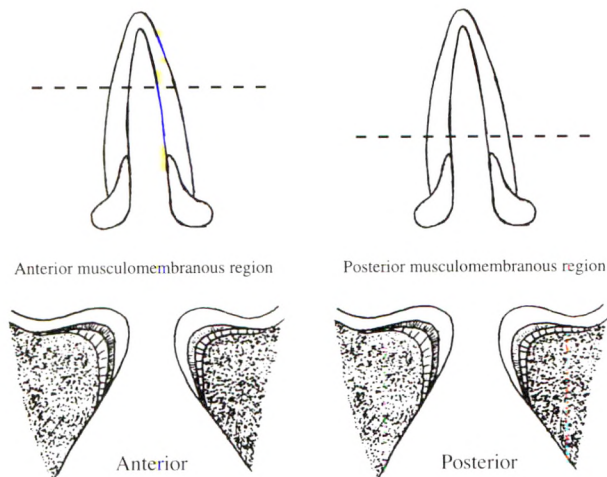


Fig 1. Anatomic map used during surgery, delineating 3-dimensional characteristics of vocal edge lesions in relationship to epithelium and laminae propria. Muscle is designated by stippled area, while vocal ligament medial and deep laminae propria are noted by 2 thinner layers superficial to muscle. Superficial lamina propria (SLP) is white, unshaded area deep to epithelial membrane, which is designated as dark line at surface.

sess overall outcomes in a consecutive series of performing vocalists who were managed by a multidisciplinary team and for whom the primary treatment was surgery. This study was catalyzed by the observation that there had been a gradual growth in the number of singers who were deemed surgical candidates. This philosophy evolved from the expanding indications for and successful outcomes (subjective and objective) of surgery. The need for this investigation is illustrated by the fact that a search of the English-language literature since 1968 only uncovered 2 reported series (in peer-reviewed literature) of singers undergoing microsurgery.^{25,29} It was our hope that information derived from the investigation of vocalists would 1) shed light on key management issues that could be extrapolated to the general population and 2) identify pertinent unanswered questions to stimulate future research initiatives.

MATERIALS AND METHODS

PATIENT AND LESION CLASSIFICATION

We examined prospectively gathered data on a consecutive series of 185 performing artists who underwent suspension microlaryngoscopy and phonomicrosurgical resection for glottic disorders. Inclusion criteria were 1) that the patient indicated that he or she was seeking laryngological management for enhancement of his or her singing or performing voice and 2) that the patient and surgeon cooperatively determined that a vocal fold procedure was judged to be integral to the treatment. All of the performers operated on between January 1994 and January 2002 who did not present with a sulcus defor-

mity as the primary indication for microlaryngoscopy were included in the study. There were 4 patients in the sulcus group.

Patients reported on their primary voice activity or style by means of a questionnaire: classical, pop or rock, jazz or blues, gospel, country, folk, and musical theater. Two other classifications included broadcasters and a singing clown. At the time of the surgery, the microlaryngoscopic examination of the disorder was recorded in an operative data collection form, which included a map (Fig 1) to delineate the disorder and its relationship to the epithelium and laminae propria. Phonotraumatic lesions were classified during the operation by Dikkers³⁰ criteria (nodule, polyp, cyst, keratosis, granuloma, polypoid corditis, varix, or ectasia). Further criteria for other lesions were extrapolated from a work by Zeitels.²⁸

SURGICAL METHODS AND VOCAL REHABILITATION

The timing of surgery and the extent of preoperative voice therapy were individualized on the basis of a number of factors, including lesion characteristics, concomitant inflammation, hemorrhage, medical illness, and geographic proximity of the patient's home. The preferences of the patient, voice therapist, and surgeon were also contingent factors. Finally, performance and recording schedules frequently delineated the window of opportunity for comprehensive intervention. Almost all patients underwent postoperative voice therapy, and this was individualized on the basis of the aforementioned factors.

All procedures were performed under general anesthesia with the smallest endotracheal tube that would allow for adequate ventilation. Typically, the intubation was done by the operating surgeon. The largest tubular triangular glottiscope^{31,32} that could be placed from the lips to the glottis was used.²⁷ All patients were placed in the Jackson position³³⁻³⁵ with a true suspension gallows (Endcraft LLC) used to achieve elevated vector suspension.²² External counterpressure³⁶ was applied with silk tape and, at times, with a laryngeal cushion.^{9,34} The details of the technique have been previously described.^{9,22,34}

Except for 3 patients with ectasias or varices from the early part of the series,³⁷ all resections were performed with cold instrumentation.²³ All of these phonomicrosurgical procedures were primarily done at the highest magnification on the microscope. This was 10× until 1998 and was then 13× after a more powerful microscope was obtained. The effective magnification is not reflected by the numbers on the microscope dial, since a 400-mm front lens is used. A subepithelial infusion^{20,22,27,28,38} of saline solution with 1/10,000 epinephrine was done in most cases.

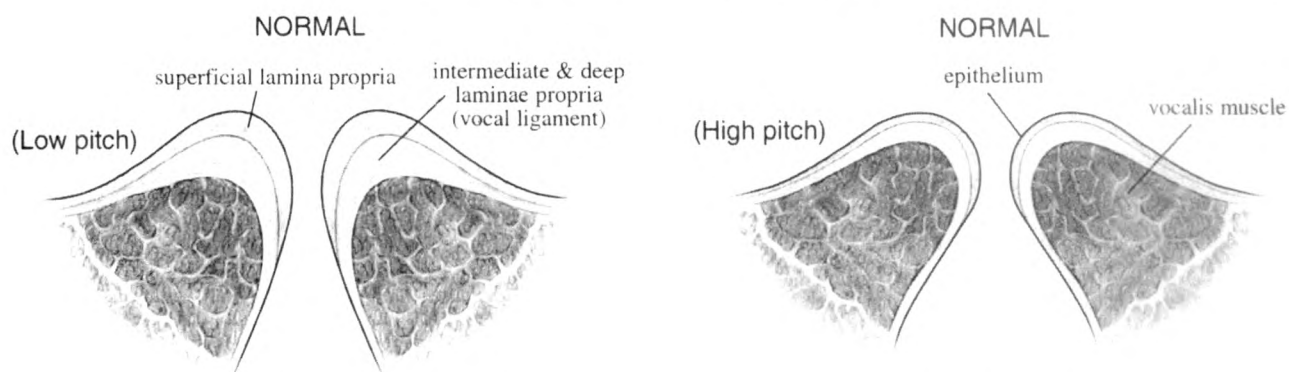


Fig 2. Coronal-section diagrams of normal vocal folds during vibration at low pitch and high pitch.

The infusion enhances preservation of residual normal superficial lamina propria (SLP) by 1) facilitating the epithelial cordotomy, 2) expanding the SLP, thus reducing instrumental trauma to it, 3) improving visualization by vasoconstriction of the microcirculation, and 4) improving the visual and palpable interface between the lesion base and the underlying SLP. The infusion was used in all cases except those in which it was deemed that the fluid would obscure the visualization of very small lesions (1.5 to 0.2 mm). The guiding principle of the surgical approach was to not disturb any tissue that was deemed normal by highest-power examination and palpation.

An epithelial cordotomy was made at the superior-lateral aspect of the lesion in most cases. In the case of nodules and polyps, the fibrovascular tissue is adherent to the epithelium from long-term basement membrane zone trauma.³⁹ These clinical observations are commensurate with the immunocytochemical findings on nodules and polyps by Gray et al.⁴⁰ Therefore, once the epithelial cordotomy is performed, blunt and sharp dissections are done at the interface between the fibrovascular tissue and the underlying normal residual SLP. Identification of this dissection plane is enhanced by the subepithelial infusion, which increases the visual and textural contrast between the normal SLP and the overlying lesion. Since nodules and polyps have normal overlying epithelium, the membrane is preserved by a subepithelial resection.^{28,37,41,42} It is our belief that generally, medial surface keratosis in nonsmoking singers need not be resected, since it will revert to non-keratinizing epithelium once the vocal edge is straight and the excessive collision trauma abates. Our impression is that maximal preservation of the normal underlying SLP and overlying epithelium accelerates healing and leads to an optimal result. Schematic representations of normal vocal fold anatomy (Fig 2), as well as a number of lesions (Figs 3A, 4A, and 5A), are depicted. Resections of each type of lesion (polyps, ectasias, varices, and cysts, as well as nodules, which were in the majority) are seen in Figs 3-

6.³⁷ Reinke's edema⁴¹ was surgically managed by performing a superiorly located cordotomy and then extracting the gelatinous subepithelial material. Topical epinephrine was applied as necessary for hemostasis.

The patients were maintained on complete voice rest for a minimum of 10 days after the surgery. The surgeon performed stroboscopic examinations at approximately 2 weeks to assess mucosal pliability as part of a routine postoperative office visit. If the postoperative stroboscopy demonstrated proper healing, the patient was allowed gradual resumption of voice use under the guidance of a speech pathologist. All patients underwent antireflux management with behavior and diet control and proton pump inhibitors once or twice per day during the perioperative period.

ASSESSMENT OF VOCAL FUNCTION

The standard protocol included complete voice assessments before and after surgery and after the completion of voice therapy (typically 2 months after surgery). A complete assessment included both subjective and objective measures.

SUBJECTIVE MEASURES

Videoendoscopy With Stroboscopy. Standard transoral rigid (Kay Elemetrics 70°) and/or transnasal flexible (Olympus ENF P-3) videolaryngoscopy with stroboscopy was performed as each patient sustained /i/ vowels at comfortable (average) and increased pitch and loudness levels. These examinations were recorded on videotape (SVHS format) or digital video disk to enable playback for detailed evaluation. The examining laryngologist subjectively evaluated parameters related to arytenoid movement, integrity of the epithelial surface and contour of the vocal fold, glottal closure pattern (glottal gap), mucosal wave activity, and compression of supraglottal and glottal structures. Supraglottic compression was evaluated by flexible fiberoptic examination.

Our previous work has demonstrated the tendency for videostroboscopic ratings to be an unreliable ob-

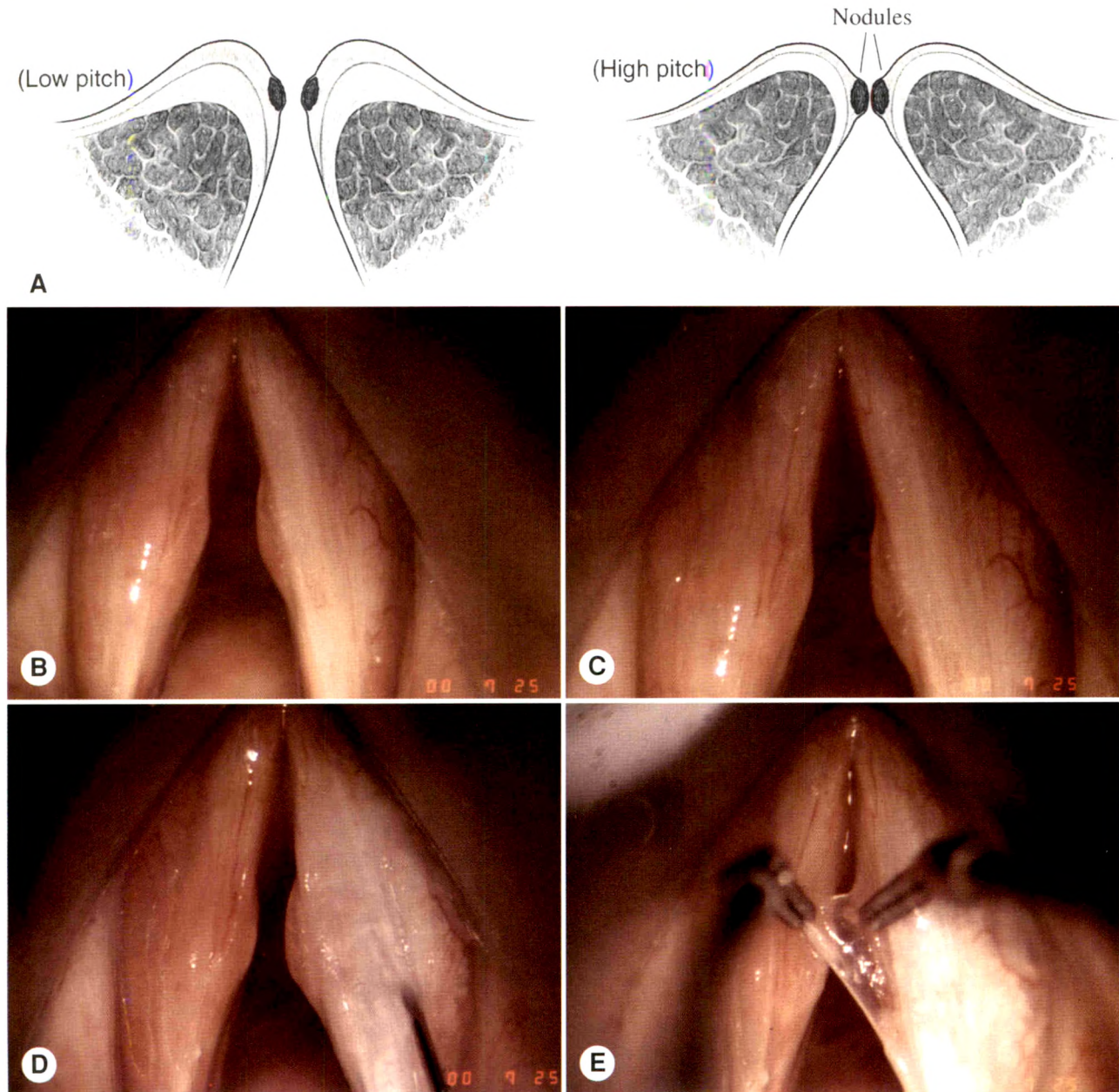


Fig 3. Pop vocalist, 38 years old, who has had progressive vocal deterioration. There has been progressive and gradual loss of timbre, range, and stability through last decade. **A)** Coronal-section diagrams of vocal folds during vibration at low pitch and high pitch. Nodules become more distinct visually in high-pitched phonation, since there is significant component of lesion within SLP, which is not easily seen during low-pitched gesture. This leads to inefficient and more disordered oscillation at higher frequencies. **B)** Exposure with universal modular (UM) glottiscope demonstrates fibrovascular nodules in midmusculo-membranous region on both sides. Wide exposure of glottis, including ventricular mucosa and superior surface of anterior commissure, facilitates positioning of lesion in center of scope. This allows for enhanced manual manipulation of soft tissue. **C)** Lesions are seen under higher magnification. **D)** Subepithelial infusion is done in right vocal fold. **E)** After epithelial cordotomy, subepithelial resection of fibrovascular tissue is done. (Continued on next page.)

jective measure of vocal outcome.⁴³ Therefore, we have taken the position that such ratings must attain an acceptable level of reliability across multiple independent judges to be used as valid research-quality data. Given the large number of patients in this investigation, it was not feasible to submit the videostroboscopic recordings for independent analysis by multiple judges. Thus, the videostroboscopic as-

sessments were not used as formal data, but are instead referred to descriptively in the Discussion to 1) reinforce important points about the pathophysiological characteristics and surgical management of the types of vocal fold lesions that were treated and 2) emphasize the critical importance and appropriate use of videostroboscopy in the clinical management of professional voice users.

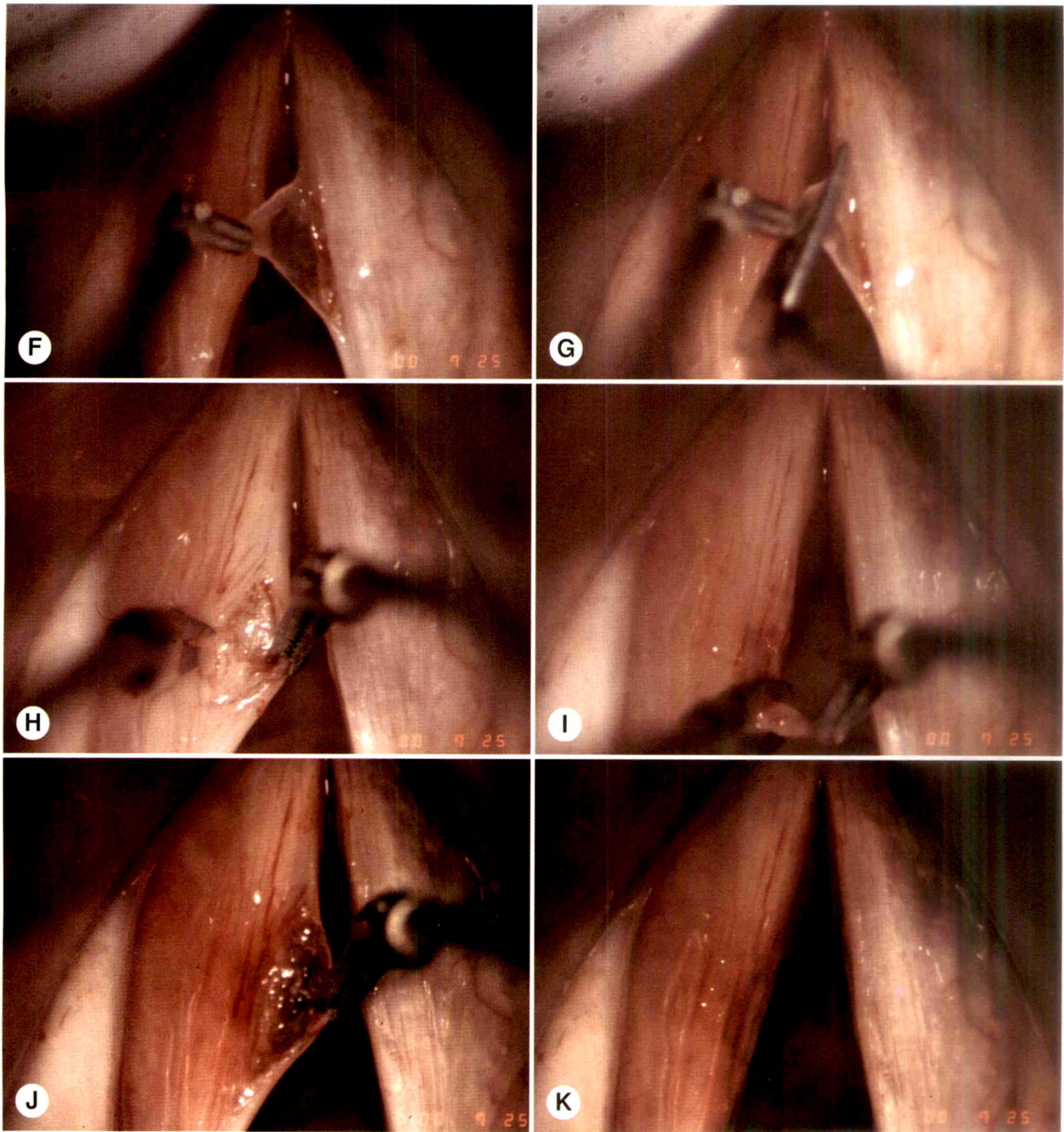


Fig 3, continued. **F)** After removal of subepithelial fibrovascular tissue, inferiorly based epithelial microflap is retracted. Note transparent and translucent nature of epithelium. **G)** Once subepithelial mass has been removed, there is redundant epithelium. Strip of epithelium (1.5 mm) is excised from superior aspect of microflap. **H)** Surgical dissection is based on identifying interface of lesion and SLP, which is enhanced by subepithelial infusion (see D). After subepithelial infusion on left and epithelial cordotomy, subepithelial fibrotic tissue is sharply dissected from underlying normal SLP. Note that centrally placed lesion allows for enhanced visualization of distal part of instruments and enhanced retraction of soft tissue. **I)** Subepithelial fibrosis has been separated from overlying epithelium, and it is resected sharply. **J)** Inferiorly based epithelial microflap is retracted. Subepithelial fibrosis has been removed, and there is maximal preservation of normal underlying SLP and overlying epithelium. **K)** Vocal folds are now smooth and straight, with excellent apposition of epithelial edges so that epithelial cordotomies are barely visible.

Patient Postsurgical Self-Assessment. At the time of the postsurgical assessment by the laryngologist, each patient was asked to respond to the question, "How is your voice now as compared with how it

was prior to surgery?" The patients were required to respond using a 3-interval forced-choice scale: 1 = same, 2 = worse, and 3 = better. The patients were carefully advised to return for reexamination if their

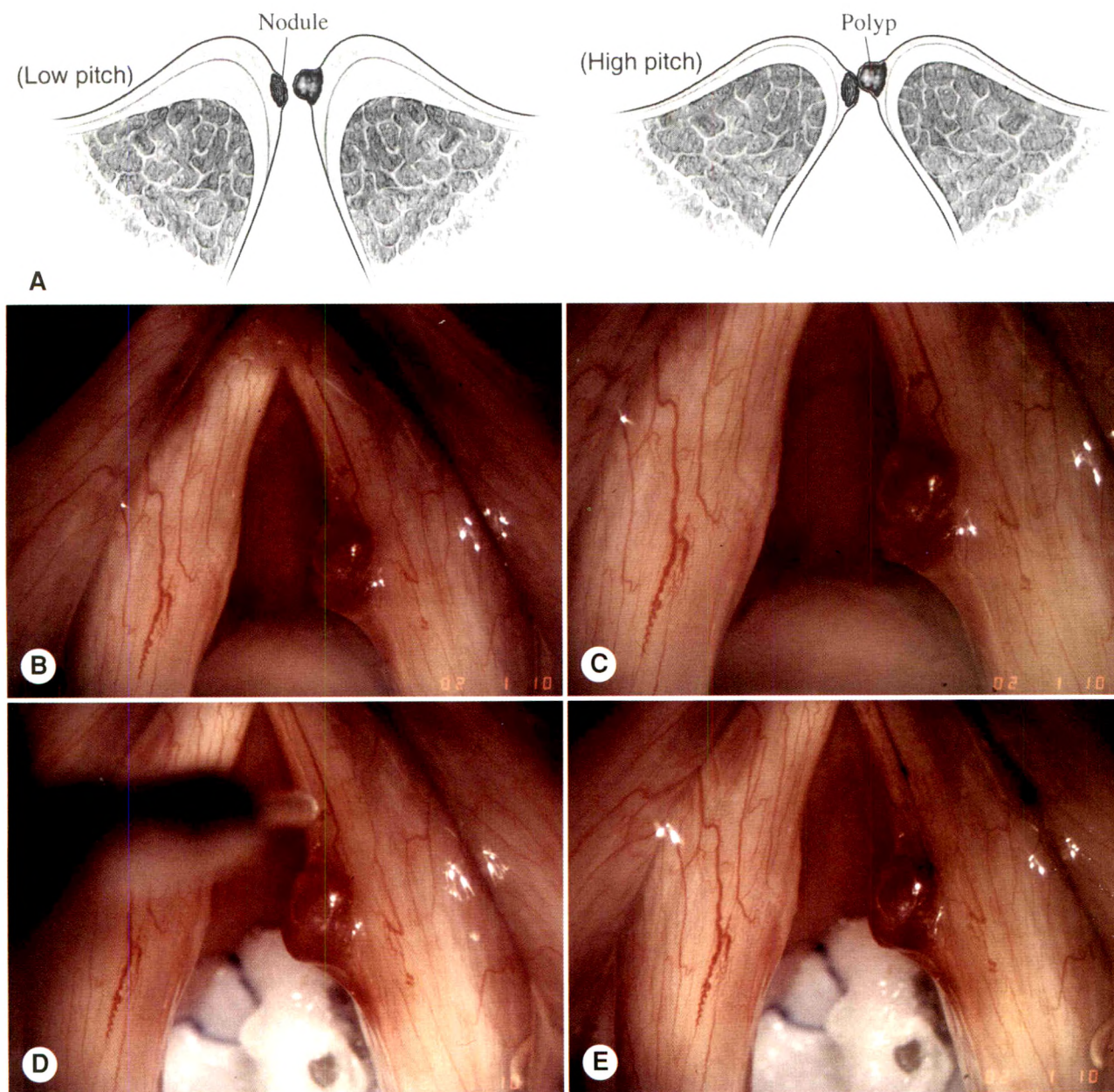


Fig 4. Broadway musical theater performer, 37 years old, has had severe recurrent hemorrhage in right vocal fold. Despite some vocal deterioration over past 5 years, she is singing well. **A)** Coronal-section diagrams of vocal folds during vibration at low pitch and high pitch. There is less functional SLP underlying lesions at high pitch, which leads to inefficient and more disordered oscillation. **B)** Remarkably, dysphonia is not her primary complaint, despite obvious phonotraumatic lesions. **C)** There is 3.5-mm fibrovascular polyp on medial aspect of right musculomembranous vocal fold. There is prominent varix noted anterior to it. There are multiple prominent varices on superior surface of left midmusculomembranous vocal fold. On medial surface, there is fibrovascular nodule. **D)** Fiber from 585-nm pulsed dye laser can be seen directed at prominent varix. **E)** After use of pulsed dye laser, feeding vessel demonstrates dark color due to photoangiolytic and intravascular coagulation. (Continued on next page.)

vocal quality deteriorated after the final voice evaluation.

OBJECTIVE MEASURES

Acoustic Assessment. Each patient was seated in a sound-treated room (IAC booth) and wore a head-band device that suspended an electret condenser microphone a constant distance of 15 cm from the lips. The output of the microphone was fed to a preampli-

fier and then recorded on digital audiotape. A calibration tone of known intensity (decibels sound pressure level [SPL]) was recorded before each patient evaluation and used as a reference for acoustic analysis. Recordings were made as each patient performed the following tasks: 1) read a standard passage (the Rainbow Passage), 2) sustained the vowel /a/ 3 times holding pitch and loudness as "steady" (constant) as

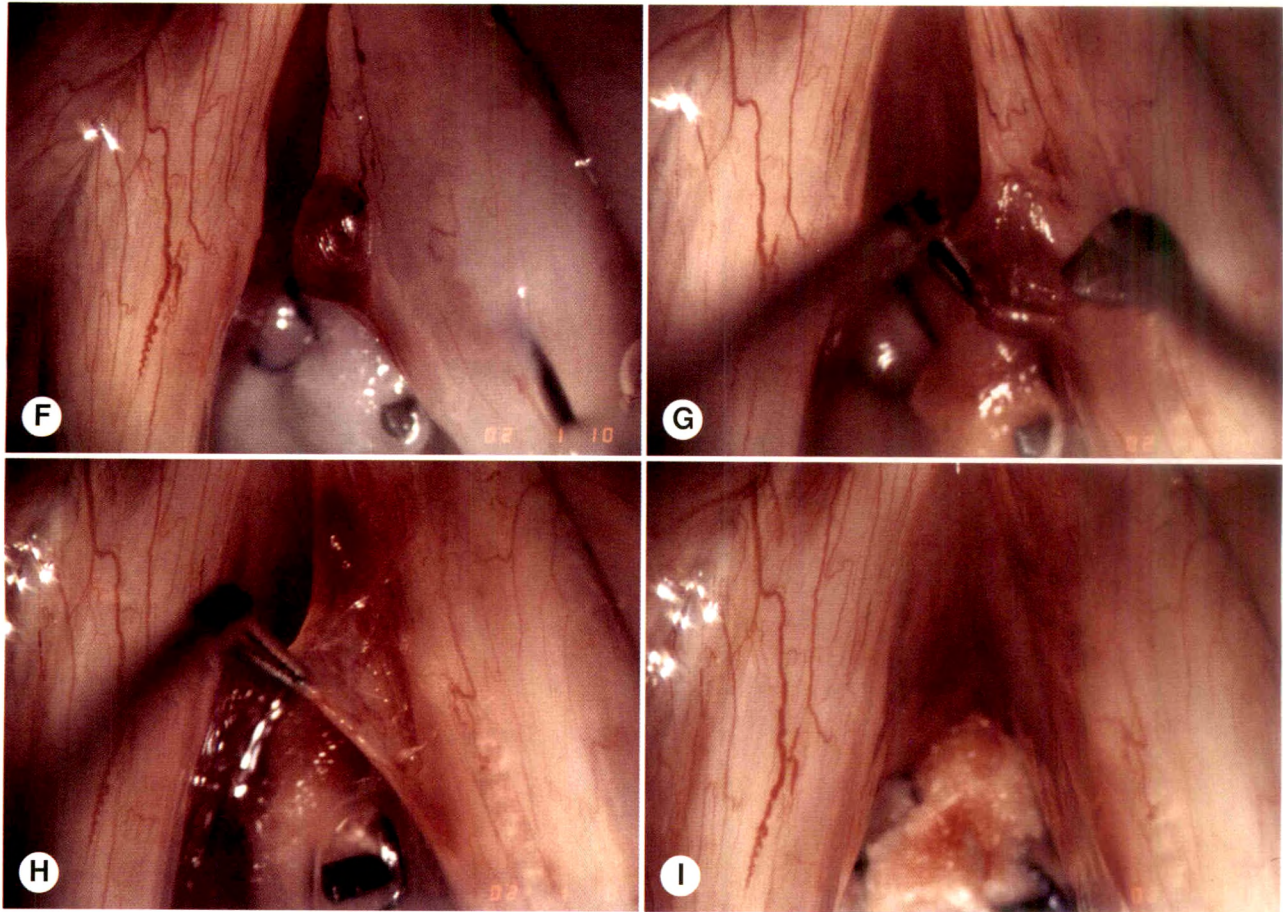


Fig 4, continued. **F)** Subepithelial infusion is now done within SLP of right vocal fold. Note extensive distention that can be achieved with saline solution because of unusually large volume of SLP. This anatomic characteristic of SLP, which is associated with musical theater performance (belting), has allowed her to perform despite bilateral mass lesions of her vocal folds. **G)** After epithelial cordotomy, microflap is retracted with curved alligator forceps, and mini-micro scissors is used to create dissection plane at interface between fibrovascular mass and normal underlying SLP. Surgical dissection is based on identifying interface of lesion and SLP, which is enhanced by subepithelial infusion (see F). **H)** Subepithelial resection of vascular mass has been done. There is slight vascular staining of SLP anteriorly, which is result of dissection of subepithelial mass. **I)** There is excellent coaptation of epithelial edges so that cordotomy incision is barely perceptible. Afterward, lesion on left vocal fold was resected.

possible for at least 3 seconds, 3) sustained the /a/ vowel 3 times at a comfortable level for as long as possible on 1 breath, and 4) produced the /a/ vowel at the “softest” (without whispering) and “loudest” sustainable levels possible 3 times each, and produced the /a/ vowel at the “highest” and “lowest” sustainable pitches possible 3 times each. The audio recordings of the reading passage (10-kHz sampling rate) and vowels (20-kHz sampling rate) for each patient were digitized and processed on a Pentium II/300 PC workstation with software that is commercially available (Computer Speech Lab, Kay Elemetrics, Inc). The reading passage was analyzed to yield measures of average fundamental frequency (F0) and intensity (decibels SPL). The steadiest 1-second segment from the midportion of each of the /a/ vowels produced for 3 seconds was analyzed to yield the percent frequency perturbation (jitter), the percent intensity perturbation (shimmer), and the

noise-to-harmonics ratio. The maximum phonation time was determined from the longest sustained /a/ vowel. The maximum intensity range was obtained by analyzing the intensities for the vowels produced with “softest” and “loudest” sustainable phonations (maximum range equals highest intensity minus lowest intensity). The maximum frequency range was obtained by analyzing the F0 (hertz) for the vowels produced with the “highest”- and “lowest”-pitched phonatory gestures (maximum range equals highest F0 minus lowest F0).

Electroglottographic Assessment. Electroglottographic recordings were obtained simultaneously with the acoustic recordings by means of a neck band that held surface electrodes against the skin over the thyroid lamina of the larynx. The electroglottographic signals obtained during sustained (3 second) vowel production were analyzed to yield an average duty

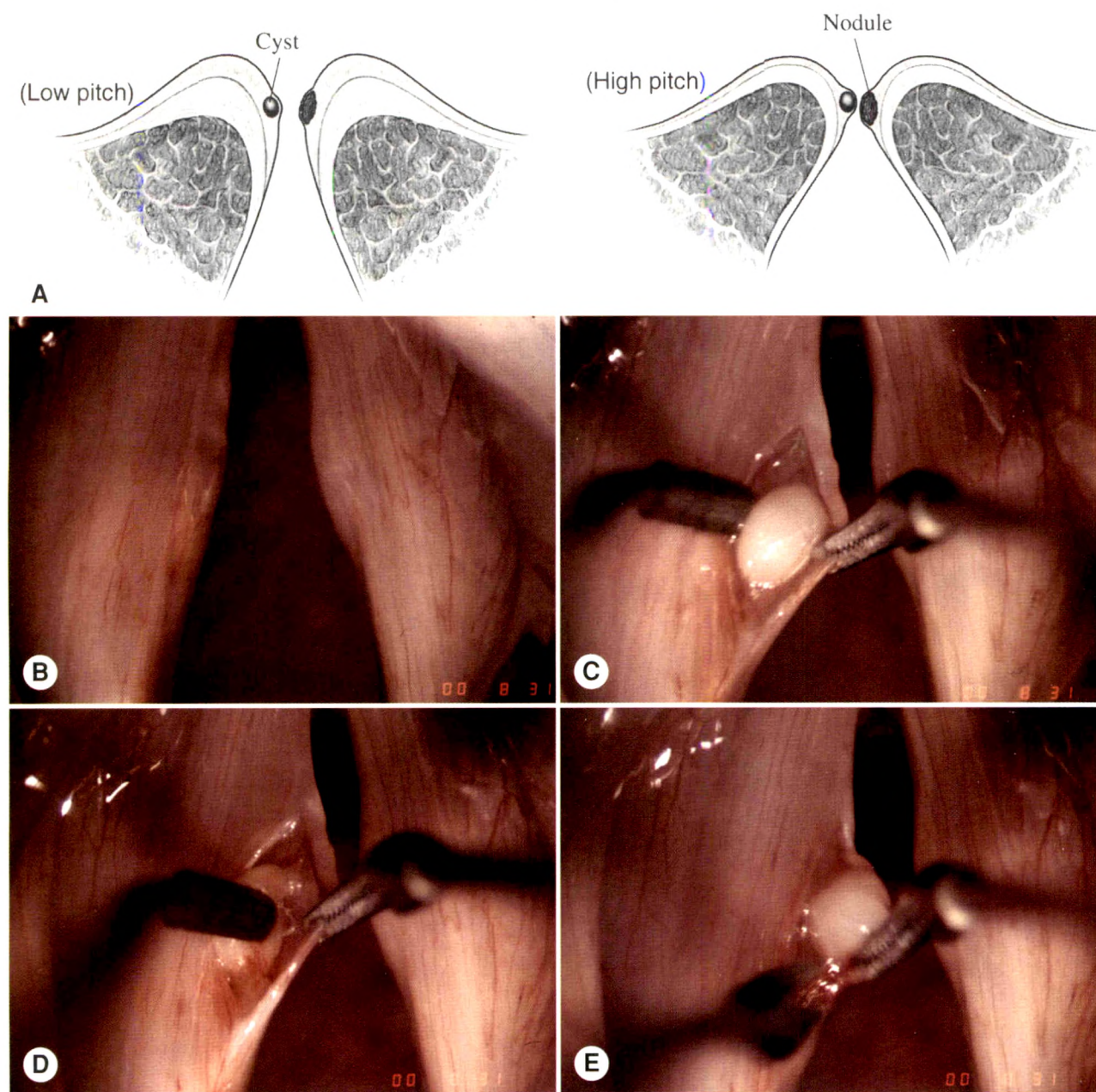


Fig 5. A) Coronal-section diagrams of vocal folds during vibration at low pitch and high pitch. Similar to conditions in 3A and 4A, there is less functional SLP underlying lesions at high pitch, which leads to inefficient and more disordered oscillation. B) Subepithelial infusion has been performed on left vocal fold, and one can see white-appearing mass through transparent epithelium near medial surface. There is very minimal contour change from mass in medial surface of left vocal fold. On right, there is fusiform prominence of medial surface of midmusculomembranous vocal fold. C) Surgical dissection is based on identifying interface of lesion and SLP, which is enhanced by subepithelial infusion. After epithelial cordotomy, epithelial microflap is retracted medially and blunt dissector is positioned between cyst and underlying normal SLP. Note angulation of hand instruments and centrally located lesion. D) There is some fibrous adherence of cyst to overlying epithelial basement membrane. Cyst is retracted with blunt dissector, and fibrous attachments can be seen. E) Area of fibrous attachment (1 mm) is maintained posteriorly as means of retraction. It is sharply dissected from residual normal SLP with micro scissors. (Continued on next page.)

cycle measure (duty cycle equals time vocal folds are apart divided by period of vocal fold vibration).

Aerodynamic Assessment. Aerodynamic testing was done with an Aerophone system (Kay Elemetrics, Inc). The patients produced 3 strings of 5 /pae/

syllables at both a comfortable (normal) level and a loud (as if to be heard across the room) level while the following measures were obtained: 1) oral airflow measured with a pneumotachograph mounted in a face mask and 2) intraoral air pressure measured with a translabially placed catheter connected to a

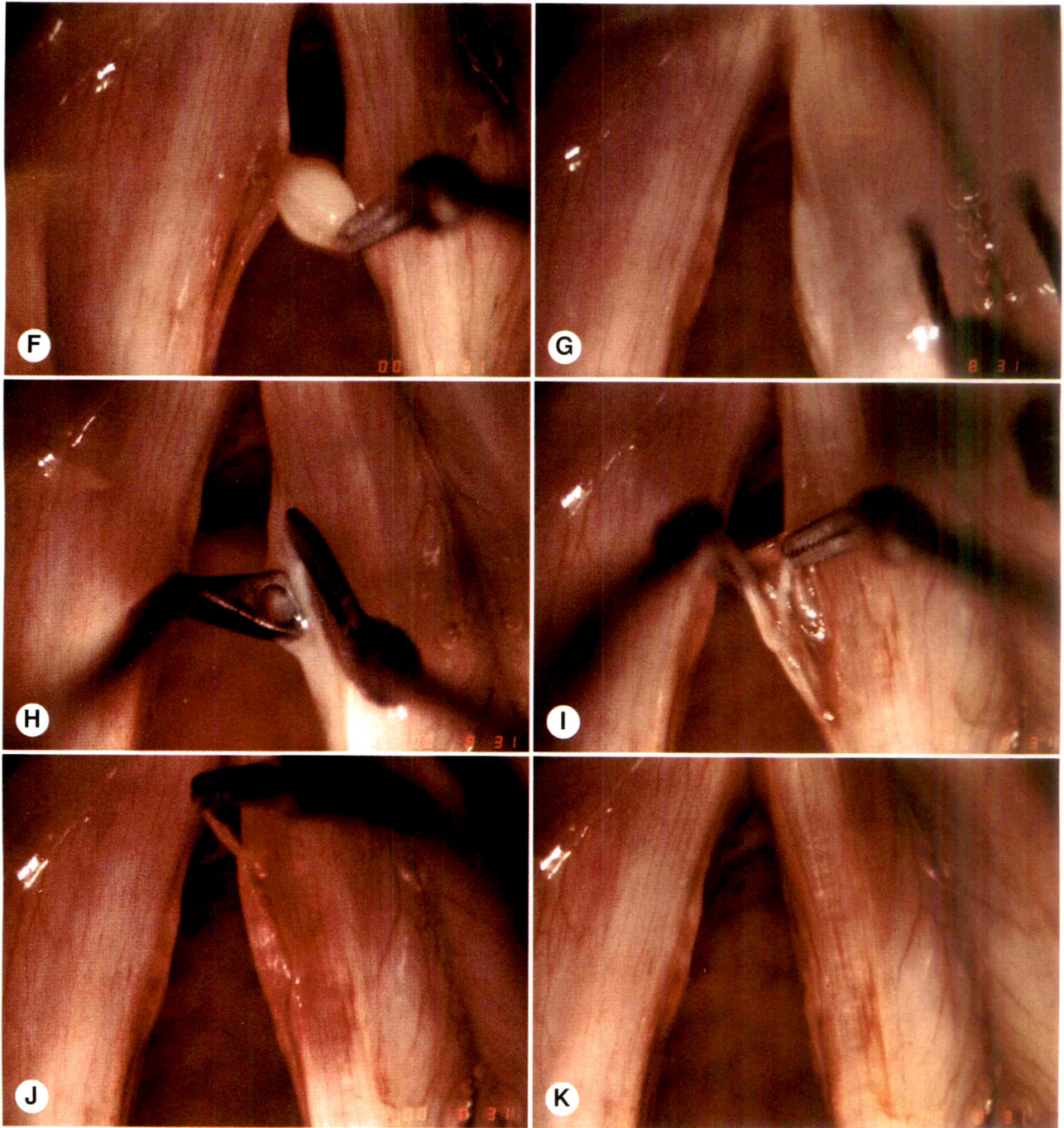


Fig 5, continued. **F)** Posterior fibrous attachment allows for retraction of cyst without rupture so that it can be withdrawn from epithelial cordotomy. **G)** Subepithelial infusion is done in right vocal fold. Note translucent nature of most of vocal fold and medial white area in region of fusiform mass. White area is not spheroid and suggests fibrotic process secondary to long-term basement membrane zone injury with associated dense fibrosis. **H)** Vocal fold is retracted, and epithelial cordotomy is done at edge of lesion. **I)** Curved alligator forceps is used to retract epithelial microflap. Second mini-micro alligator forceps was used to dissect underlying fibrosis from overlying epithelium. Fibrotic tissue, which is being retracted, had been previously dissected with mini-micro scissors. Again, notice extreme angulation of instruments, which enhances retraction and visualization, and which is facilitated by lateral slots in UM glottiscope. **J)** Portion of fibrous tissue is withdrawn through epithelial cordotomy. **K)** Right vocal fold is now smooth and straight, after subepithelial resection of phonotraumatic fibrosis. On left, there is slight edema of flap, which will resolve over several days and which should not be resected. Again, there is excellent coaptation of epithelial edges so that cordotomy incisions are barely perceptible.

pressure transducer. The flow and pressure signals were digitized and analyzed by the Aerophone system to yield indirect estimates of average glottal air-

flow rate (liters per second) and subglottal air pressure (centimeters of water). The middle 3 syllables of each string were analyzed to yield 9 measures per

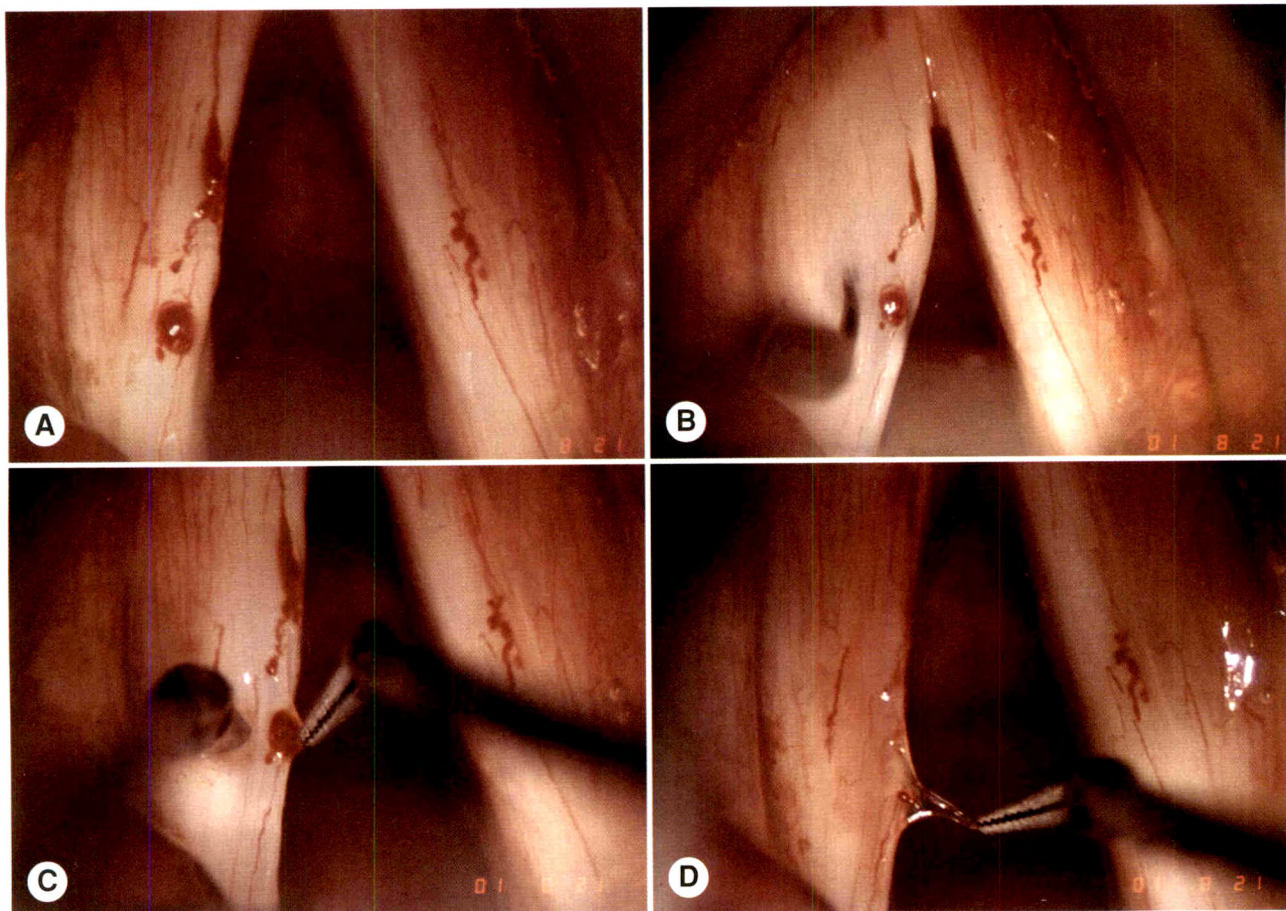


Fig 6. Musical theater performer, 49 years old, who has had extensive Broadway career and recurrent vocal hemorrhages. In addition, she has developed vocal instability that begins after approximately 30 minutes of performance. **A)** There are multiple phonotraumatic ectasias and varices generally located on superior surface of midmusculomembranous vocal folds. **B)** Subepithelial infusion of saline solution and 1/10,000 epinephrine is done on left. **C)** After epithelial cordotomy, epithelium is grasped with curved alligator forceps to allow for sharp dissection of vascular mass. **D)** Subepithelial resection of mass is performed with maximal preservation of overlying epithelium and underlying SLP. Note that only last serration of mini-micro alligator forceps is used to retract subepithelial soft tissue. (Continued on next page.)

loudness condition that were then averaged to produce 1 data value per condition.

RESULTS

The distribution of the primary vocal activities among the cohort of performers is shown in Table 1. The ages of the patients ranged from 16 to 62 years, and the median was 32 years. The distribution of the lesions that were encountered is displayed in Table 2. Postoperative subjective assessment rating by the patients revealed that 182 of the 185 thought that their performing voice was improved. Three of the 185 described no change (1 of 3 had extensive resection of keratosis with atypia), but no patient reported deterioration of his or her voice. All patients returned to full vocal activities. Complete postoperative voice assessments with objective measures were available for 127 of the 185 individuals. Although all patients underwent preoperative objective assessment, many patients were not available for a complete long-term postoperative evaluation because of performance

scheduling and geographic inconvenience.

The average presurgery and postsurgery values for the acoustic and aerodynamic measures of vocal function that are gender-sensitive are shown separately for the male and female patient groups in Table 3. In the male group, the only measures that reflected presurgical abnormality were average SPL for reading and maximum phonation time. After surgery, there were no abnormal values; moreover, there was a significant increase in maximum phonation time ($p < .05$, simple t -test). In the female group, there were no presurgical abnormalities. After surgery, there were no abnormal values, and there were increases in maximum phonation time, average F0 for reading, maximum F0, and maximum F0 range ($p < .05$, simple t -test).

A comparison of the average presurgery and postsurgery values for the remaining measures of vocal function for the entire patient group is shown in Table 4. Presurgical abnormalities were reflected in an ele-

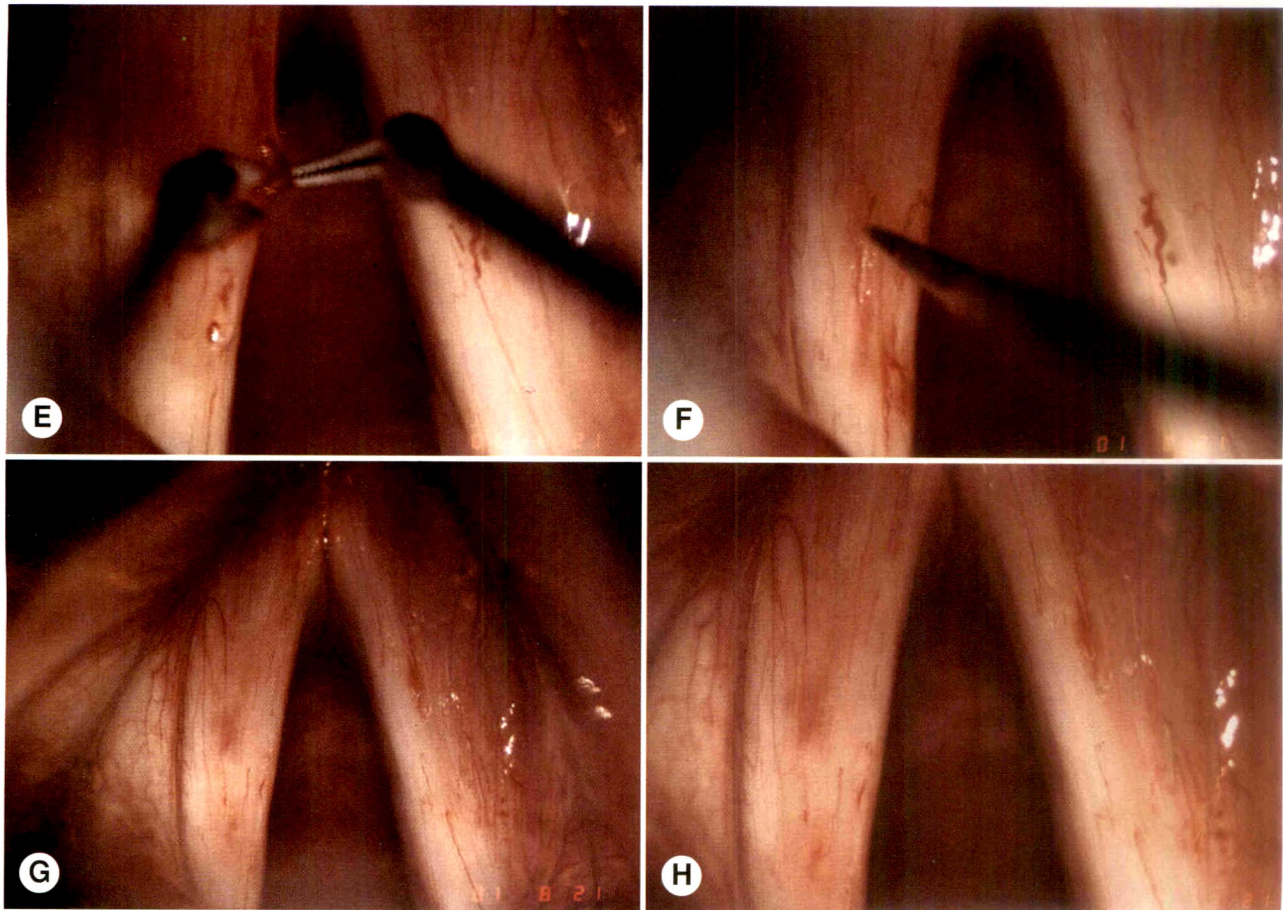


Fig 6, continued. **E)** Similar procedure is done on more anterior phonotraumatic vascular anomaly. Examining speculum of UM glottiscope allows for lesion to be centered in lumen of scope. This fact, along with lateral proximal slots, allows for excellent angulation of instruments and in turn retraction of epithelial microflaps. **F)** Sharp pick is employed to dissect out superior-surface varix. **G)** On lower power through UM glottiscope, one can see that vascular malformations have been resected by means of cold instruments. Lancet-arch conformation of scope allows for maximal visualization of glottis. Superior surfaces of vocal folds are visualized well into ventricle and on superior surface of anterior commissure tendon. **H)** Image in G, seen in higher magnification. Note that vascular lesions have been cleared from "striking zone,"³⁷ and multiplicity of epithelial cordotomies is difficult to perceive, since there was no significant loss of epithelium or layered microstructure.

vated percent shimmer for sustained vowels and an elevated subglottal pressure in men for comfortable voice production. None of the measures were abnormal after surgery. There were significant ($p \leq .05$) postsurgical decreases in the percent shimmer for sustained vowels and the subglottal pressure for loud voice production, as well as significant increases in the SPL–subglottal pressure ratios for loud voice, which reflect an increase in the efficiency of phonation.

Almost all of the nodule patients who had surgical intervention underwent extensive vocal rehabilitation therapy with a speech pathologist (with classical singing training) and were found to have recalcitrant lesions (Figs 3 and 5) before phonomicrosurgical intervention.⁴⁴ During the operation, they were visually observed to have fibrovascular changes that were often adherent to the epithelial basement membrane (Fig 5I).

Ten of the 185 patients required a second surgical procedure. All 10 are vocally improved, singing actively, and very satisfied with their outcome. Complete presurgical and postsurgical data were available in 7 of the 10 patients subsequent to the second procedure. Four of the 10 (2 pop or rock, 1 classical, 1 musical theater) developed recurrent vocal nodules meriting a second procedure between 1 and 2.5 years after the first. Two of the 4 continue to have mild nodular changes but are singing well, and their voices remain better than at their preoperative presentations. Two of the 10 (1 pop or rock, 1 jazz or blues) abused their voices approximately 3 days after operation and required an immediate second procedure to eradicate a new vascular lesion. Two of the 10 (pop or rock) developed a new polyp 1.5 and 2.5 years later. One of the 10 (broadcaster) has papillomatosis. One of the 10 (classical) was noted to have a residual fibrovascular nodule in the early postoperative period. Although the patient had formidable

TABLE 1. PRIMARY SINGING STYLE OR VOCAL ACTIVITY

	No. of Patients
Classical	82
Pop or rock	44
Musical theater	26
Jazz or blues	9
Folk	8
Gospel	7
Broadcaster	5
Country	3
Clown	1
Total	185

coughing in the immediate postoperative period, most likely the initial ultraconservative resection failed to entirely remove the lesion. The residual nodule was approximately 25% of its size at original presentation and was reexcised with an excellent outcome. Three of the 185 patients only partially followed (approximately 1 week) the voice rest protocol and had no untoward effect. Therefore, 182 of the 185 reported following the voice rest protocol successfully.

DISCUSSION

Phonomicrosurgery in performing artists has historically been associated with controversy because of variable results, as well as divergent opinions about the pathogenesis of lesions and the potential non-surgical reversibility. Furthermore, the tremendous societal visibility of performers leads to significant publicity both in and out of the entertainment industry if suboptimal surgical outcomes occur. Paradoxically, excellent results are often obscured by the artists' desire to conceal the injury and surgery for fear it will have a career-damaging affect. This fear arose from a commonly held misunderstanding in the entertainment industry that the need for surgical intervention suggests compromise or a flaw in the performer. In fact, the sustained injury or lesion is fre-

TABLE 2. DISTRIBUTION OF LESIONS ENCOUNTERED

Lesion	No.
Nodules in 119 patients (24 male, 95 female)	201
Polyps	71
Varices and ectasias	66
Cysts	13
Keratosis	8
Granuloma	2
Papilloma	2
Reinke's edema	2
Total	365

Many patients had more than 1 lesion.

quently the result of high-level professionalism that motivates a vocalist to perform despite routine illnesses. Finally, the performer often has questions and subsequent concerns that he or she has not properly taken care of this gift or talent. Unlike a dancer, who can use visual feedback (mirror) to make adjustments, the singer is dependent on more subjective methods of sensation, proprioception, and self-perception of acoustic quality. These professional and emotional issues can substantially affect the surgeon's discussions with the patient regarding the nature of the performer's lesion and the pathway for intervention and rehabilitation.

This investigation was performed to examine the results of phonomicrosurgery in vocal performing artists, since the literature is extremely sparse in this area and because there are unique circumstances and expectations related to these procedures. In terms of the important metric of patient self-assessment, nearly all patients reported improvement in their voice production subsequent to surgery. This prospective study commenced before formal patient self-assessment outcome instruments were established. Given the present mainstream acceptance of such measures, we have recently adopted this approach for future studies. Objective acoustic and aerodynamic mea-

TABLE 3. RESULTS OF ACOUSTIC AND AERODYNAMIC MEASURES FOR MALE AND FEMALE GROUPS

Measure	Men			Women		
	Presurgery	Postsurgery	p (t-test)	Presurgery	Postsurgery	p (t-test)
Average SPL, reading (dB)	68.7	71.2	.203	69.0	69.5	.639
Average F ₀ , reading (Hz)	130	134	.247	197	204	.000
Maximum SPL range (dB)	51.9	52.7	.718	52.0	49.4	.320
Maximum F ₀ (Hz)	624	616	.861	847	910	.027
Maximum F ₀ range (Hz)	535	528	.885	712	777	.025
Maximum phonation time (s)	16.7	20.1	.019	16.4	19.6	.000
Average flow, comfortable (L/s)	0.186	0.179	.697	0.189	0.175	.088
Average flow, loud (L/s)	0.210	0.212	.929	0.184	0.168	.062

Values in italics fall outside normal range; p values in bold indicate statistical significance ($p \leq .05$).

Normal values used for comparisons: average sound pressure level (SPL) for reading, men ≥ 70 dB, women ≥ 68 dB; average fundamental frequency (F₀) for reading, men 82 to 146 Hz, women 180 to 240 Hz; maximum SPL range, ≥ 40 dB; maximum F₀, no normative values available; maximum F₀ range, ≥ 2 octaves; maximum phonation time, men ≥ 18 seconds, women ≥ 14 seconds; average airflow rate for comfortable voice, men ≤ 32 L/s, women ≤ 28 L/s; average airflow rate for loud voice, men ≤ 0.38 L/s, women ≤ 0.29 L/s.

TABLE 4. RESULTS OF ACOUSTIC AND AERODYNAMIC MEASURES FOR ENTIRE GROUP

Measure	Presurgery	Postsurgery	p (t-test)
Jitter (%)	1.203	1.05	.172
Shimmer (%)	4.356	3.727	.002
Noise-to-harmonics ratio	0.139	0.135	.206
Electroglottographic duty cycle (%)	55.6	56.4	.273
Subglottal pressure, comfortable (cm H ₂ O)	8.3	7.9	.098
Subglottal pressure, loud (cm H ₂ O)	13.8	12.4	.000
SPL-subglottal pressure ratio, comfortable	9.6	10.1	.068
SPL-subglottal pressure ratio, loud	6.6	7.3	.000

Values in italics fall outside normal range; p values in bold indicate statistical significance ($p \leq .05$).

Normal values used for comparisons: jitter, $\leq 1.4\%$; shimmer, $\leq 3.8\%$; noise-to-harmonics ratio, ≤ 0.19 ; electroglottographic duty cycle, 55% to 65%; subglottal air pressure for comfortable voice, 3.3 to 8.1 cm H₂O; subglottal air pressure for loud voice, 4.2 to 12.5 cm H₂O; SPL-subglottal air pressure ratio for comfortable voice, 8.6; SPL-subglottal air pressure ratio for loud voice, 6.1.

ures were obtained on a majority of patients, and this is the first time this type of data has been reported in the English-language literature.

It is not surprising that objective measures could not be obtained on a sizeable number of patients (approximately 30%) after operation. It is common for performers to be noncompliant with detailed final assessments for a variety of reasons: travel distance, performance commitments, time constraints, etc. This cohort is unlike oncology follow-up subjects, who are geographically stable and in whom it is suspected that missing subject data suggest poor survival, the primary metric for success. Poor outcomes in the vocalist population would have been captured by patient responses to the postsurgical self-assessment questioning. In fact, difficulty in getting patients to return for final assessment typically indicates that they are performing successfully and are not motivated to return for an evaluation that will not alter their function.

OBJECTIVE MEASURES OF VOCAL FUNCTION

It is not surprising that only a few of the "objective" measures (Tables 3 and 4) reflected abnormalities in vocal function before surgery. Current state-of-the-art objective measures of vocal function (acoustic, aerodynamic, and electroglottographic) and standard voice assessment protocols are not at a point of development or sophistication that allows for the valid and reliable evaluation of typical higher-level vocal performance-related factors, eg, changes in timbre or control, especially during a light *pas-*

sagio, inability to consistently sing high-pitched and/or soft notes, decreased stamina, etc. These symptoms may occur at any singing encounter or more generally may affect one's ability to complete a tour or performance schedule.

All objective vocal function measures fell within normal limits after operation, including the few that demonstrated presurgical abnormalities. However, given the relative insensitivity of standard objective vocal function measures to higher-level vocal performance-related factors, it is even more noteworthy that so many of the objective measures displayed statistically significant postsurgical changes (total of 8 measures) reflecting postsurgical improvements in vocal function. In addition to a significant reduction in amplitude perturbation for the entire group (from abnormal to normal), such changes in measures mostly reflect an overall improvement in the efficiency of voice production. This improvement was demonstrated by increases in F₀ range and maximum phonation time, as well as a reduction in the subglottal pressure needed to drive loud phonation.

It could be argued that other parameters provide more valid and reliable metrics for evaluating success in this patient cohort than do currently available acoustic and aerodynamic measures that do not necessarily measure the ability to sing: patient assessment of improvement or deterioration, need for further surgery, and complications. These criteria, in association with the aforementioned acoustic and aerodynamic outcome data, reflect the efficacy of surgical intervention with vocal rehabilitation for consistently enhanced vocal function.

Detailed analysis of preoperative and postoperative stroboscopy provided confirmation of the subjective assessment. Stroboscopy provides invaluable information regarding epithelial pliability when employed effectively to compare a variety of vocal gestures and conditions (ie, high-pitched and low-pressure phonation). This information can be critical for prognosticating a postsurgical result. However, stroboscopy has limited application as a research method because of its overwhelming and unavoidable subjectivity. The wide range of other shortcomings of this technique as an investigational metric has been previously delineated.⁴³

Stroboscopy yielded a number of descriptive findings that support the improvement perceived by patients. Most patients presented with an abnormal vocal fold edge before surgery, and most had a normal edge after surgery. It was not unusual to see a slight undulation of the vocal edge after removal of lesions.²⁵ However, glottal closure was dramatically improved after surgery, as were the amplitude, mag-

nitude, and phase symmetry of mucosal wave activity. The majority of patients who had smooth vocal folds before surgery presented with subepithelial ectasias and varices, and the indication for surgery was recurrent vocal hemorrhage.³⁷ Approximately one third of patients displayed postoperative asymmetry in the phase of vocal fold vibration, primarily on high-pitched phonation. This finding can be explained by the differential viscoelasticity of the vocal folds that can be secondary to hyperfunction and/or organic alteration of the SLP. The organic stiffness primarily relates to the fact that the lesion has replaced some of the SLP, and resection of the lesion cannot fully restore mucosal pliability because of the lost SLP.

Analysis of the glottal closure patterns revealed a postoperative increase in the number of patients who had a posterior chink in the musculomembranous region. Many of those who presented with an hour-glass closure pattern were left with a posterior chink. We believe this is a manifestation of persistent hyperfunction and the need for further voice therapy. The hyperfunctional behavior that results in the posterior chink may very well have been a closure pattern that was instrumental in the development of the lesion(s). We are currently investigating these concepts.

SURGICAL DECISION-MAKING

Surgical intervention in vocal performing artists presents a unique set of circumstances to the laryngologist. The patient population is typically outgoing, often quite sophisticated in regard to prior laryngological problems, and frequently outspoken about their previous care. A reticent performer is practically an oxymoron. Singers are vocal athletes and should be managed at a level of precision and sophistication commensurate with their needs. The indication for phonomicrosurgical intervention is rarely one of inherent systemic danger to the patient. Phonomicrosurgery is typically done because there is a functional performance-related deficit that is accompanied by a lesion that can be considered a major etiologic factor.

There is no exact formula for which patients should undergo surgical intervention and/or how long any patient should undergo voice therapy before surgery. The timing of surgery and the extent of preoperative voice therapy should be individualized on the basis of lesion characteristics, as well as concomitant inflammation from vocal abuse, reflux, infection, menstrual swelling, and/or smoking. Furthermore, the preferences of the patient, voice therapist, and surgeon are interdependent influences in the decision-making process. Finally, performance, recording, and traveling schedules frequently delineate the window

of opportunity for comprehensive intervention.

Patients' biases about management strategies based on their previous experiences with their performing colleagues is an extremely important consideration. The surgeon should generally avoid persuading a performer to undergo surgery. Informed consent is an extremely important issue because of the sophistication of vocalists. Surgeons must discuss the unlikely possibility that the voice can be made worse by a procedure. It is valuable to educate the patient in vocal fold physiology so that he or she can understand how surgical restructuring of the vocal fold(s) will enhance mucosal pliability, glottal closure (aerodynamic competency), and, ultimately, vocal efficiency. This was done with every patient in this series. This approach engenders a *mutual responsibility* for the decision of pursuing the elective procedure.

Patients should also be counseled that it is common to discover additional lesions during a microscopic examination under general anesthesia as compared with a clinic setting. High-powered magnification and the ability to retract the vocal folds often result in the identification of previously indiscernible abnormalities. Consent should be obtained to work on both vocal folds so that surgical judgments are not restricted during surgery unless unilateral surgery is unequivocally indicated. Finally, surgeons should relate their personal experience with the particular problem. In a performing vocalist population, unfavorable results will often become widespread information because of collegial networking among artists.

Patients should be helped to understand that they should not be unduly fearful of surgery, since many of them are performing with injured vocal folds and a procedure will often provide an opportunity for significant skill advancement.⁷ Many performers have been masking the lesion-induced vocal aberration by expending tremendous compensatory effort by means of increased aerodynamic support and muscular gymnastics.⁴⁵ In addition to acoustic vocal improvement, patients should understand that the effort of phonation may decrease and their stamina may improve, reflecting enhanced aerodynamic function and vocal efficiency.

VOCAL NODULES: SHOULD THEY BE RESECTED?

Phonomicrosurgical resection of vocal nodules is controversial, because some clinicians believe that nodules are reversible with proper vocal rehabilitation. This controversy relates partially to a lack of consistency in terminology and differences in phonatory gestures (high pitch and low pitch) during diagnostic observation. As Titze⁴⁴ has well described, the tissue changes of midmusculomembranous vocal fold

swelling are often referred to as nodules and probably are reversible. In this series, nodules were considered to be composed of permanent traumatic fibrovascular changes of the SLP.^{40,44} This fact was borne out during surgery after palpation of the nodules and by examining the SLP under high magnification after epithelial cordotomy. Additionally, almost all of the nodule patients underwent extensive vocal rehabilitation therapy with a speech pathologist (with classical singing training). Those who were found to have recalcitrant lesions with commensurate dysphonia that could be explained by the presence of these lesions underwent phonomicrosurgical intervention. Our experience has revealed that although many patients develop significant improvement in vocal function after therapy, the vast majority of lesions that we consider nodules do not involute.⁴⁶

Despite the fact that 201 of the 365 lesions were nodules, a large number of patients who presented with nodules did not undergo surgery because their function improved to an adequate level of satisfaction. Vocal rehabilitation therapy will frequently enhance performance and function so that surgery is unnecessary. One of the reasons that our surgical nodule population was high might be related to the fact that nearly half of the performers in this patient cohort were classical singers and the vocal requirements of these individuals probably require optimal anatomy. Phonomicrosurgical resection was successful in that virtually all patients were functionally better and only 5 of 119 individuals required surgery for recurrent or persistent lesions. However, this success should not be interpreted to mean that all patients developed and maintained perfectly smooth and straight vocal folds.

OBSERVATIONS ABOUT SLP ASSOCIATED WITH LESIONS IN VARIOUS PERFORMANCE STYLES

A number of consistent observations were made about the SLP of patients with phonotraumatic nodules and polyps, based on the correlation of stroboscopy and intraoperative high-magnification examination. These observations are probably the most critical development from this 8-year prospective investigation, since they suggest a new paradigm for understanding biomechanical vibration associated with vocal edge lesions and, in turn, treating them. The new paradigm has implications for the methods we employ to examine patients, assess and discuss their vocal function, and perform surgery. These observations are also critical for prognosticating patients' vocal outcome from surgery and for designing new procedures.

Most notably, when a phonotraumatic mass is seen on the medial edge of the vocal aperture, it can be

assumed that a significant component of the mass lies beneath the surface in all 3 dimensions, similar to an iceberg floating in water (Figs 3-5). In the case of vocal nodules, the surface irregularity that visually protrudes into the glottal aperture increases in higher-pitched phonation as the underlying SLP is thinned (Fig 3A). This phonatory condition leads to deterioration of vocal fold oscillation and function, which are dependent on glottal closure and epithelial pliability. Clinicians should clearly understand that functionally, the mucosa comprises the epithelium and underlying SLP⁴⁴ and that the SLP accounts for a majority of the viscoelastic properties of the vibrating mucosa. The epithelium serves mainly to passively encapsulate the SLP⁴⁴; ie, the epithelium essentially assumes the vibratory properties of the underlying SLP. Although there is vibration of the ligament-muscle complex (body), the primary source of the mucosal wave is the residual SLP.⁴⁷ Many individuals do not make a distinction between epithelium and mucosa, which leads to confusion in the literature and incorrect surgical management strategies.

It is interesting that in 1836, Bishop⁴⁸ deduced by means of anatomic dissection that the vocal membrane was the primary oscillator responsible for laryngeal sound production. This finding was prior to the existence of the field of laryngology and its advantage of observation using mirror laryngoscopy. In his seminal work entitled "Experimental Researches Into the Physiology of the Human Voice," he stated, "The true vibrating surface of the glottis is the mucous membrane. The vocal cords confer on it the tension, resistance, position, and probably other conditions necessary for vibration."⁴⁸ It seems reasonably safe to assume that Bishop is referring to the dense ligament and underlying muscle as the vocal cord and to the overlying mucosa as defined by Titze⁴⁷ as the membrane.

When a mass lesion on the medial surface of the vocal fold disrupts glottal closure, vibration is impaired because of deformation and restriction of mucosal wave propagation, as well as air leakage. When there is adequate glottal closure, effective vocal fold oscillation is predicated upon the pliability of the remaining normal SLP underlying the abnormal (stiff) fibrovascular lesion. This concept is demonstrated when we encounter patients who have large lesions and yet relatively normal conversational voices. Conversely, a patient who has a straight vocal edge and a subepithelial cyst in the midmusculomembranous region (replacing the SLP) typically has very poor vocal quality. These individuals have great difficulty modulating the glottal airstream because of the severe differential viscoelasticity of the SLP between

the vocal folds and within the involved fold. On the basis of stroboscopic observations of a variety of lesions, we believe that optimally entrained vocal fold oscillation is enhanced by symmetry of viscoelasticity. This principle is illustrated by the diplophonia that occurs when an individual with a paralyzed vocal fold attempts loud phonation, which overdrives the flaccid side.

The physiological importance of the residual SLP can also be demonstrated in almost any individual with a polyp and/or nodule. During phonatory gestures at lower frequencies, the residual normal SLP, which is adjacent to the vocal ligament and deep to the lesion, is still functioning. Typically, if vocal intensity is held relatively constant or reduced, elevating pitch leads to thinning of the underlying SLP as the thyroarytenoid muscle is tensed, and thus better visualization of the lesion. Ultimately, a pitch is reached at which the voice "breaks" and entrained oscillation of the vocal folds ceases. At this point the lesions may appear to "freeze" or "lock" in position, with vibrations occurring in subsections of the remaining healthy mucosa (usually out of phase with each other). Essentially, given a relatively straight vocal fold edge, vocal stability and oscillation are dependent on the ratio of pliable residual SLP to the overlying stiff lesion that is created by pathological soft tissue adjacent to the epithelial basement membrane. This mechanical failure is further exacerbated by impaired glottal closure at higher frequencies, because as the pliable SLP is thinned, the lesion protrudes into the glottal aperture, thereby inhibiting glottal closure and reducing effective valving of the airstream.

It should be noted that in selected circumstances, vocal stability can be achieved at the extreme upper end of the pitch range in individuals with sessile, stiff basement membrane zone injury. This is possible because the function of the residual SLP has been obviated and the fibrous subepithelial tissue simulates oscillation of the body (vocal ligament) of the vocal fold. In other words, in this unique scenario, symmetry is reestablished. An alternative mechanism for reaching extremely high pitches in the presence of fibrovascular lesions is for bilateral subsections of the vocal folds, either anterior or posterior to the stiff, nonvibrating region, to generate entrained oscillation.

The importance of the relationship of the SLP to the pathological tissue is critical for understanding variances in conversational-level versus performance-level vocal fold function in the presence of vocal fold lesions. We have made a number of subjective observations, based on the stroboscopic examinations, that

lead to a variety of theories.

For example, musical theater performers and gospel singers frequently appear to have "larger" vocal folds, primarily because of an increased volume of the SLP, which is reminiscent of Reinke's edema. Similar to the Reinke's edema patient, in whom a primary phonatory mechanism is elevated subglottal pressure for voice at conversational levels,⁴¹ gospel and musical theater performers sing with very high subglottal pressure (belting). Long-term high-pressure singing appears to lead to an increased volume of the SLP and elevated medial collision forces, with chronic basement membrane zone trauma and fibrosis on the medial valving surface of the vocal folds. In turn, their associated vocal mechanism is typically aerodynamically inefficient because of the increased mass and decreased pliability. This inefficiency is evident acoustically and visually (stroboscopy) when these individuals attempt high-pitched, soft-intensity (low-pressure) singing, especially through the register transition (*passaggio*). Furthermore, these individuals often have characteristically loud and low-pitched conversational voices due to the increased volume and stiffness of the SLP, which appear to be induced by performance-related factors.

Classical singers typically appear to have smaller vocal folds than their musical theater colleagues, perhaps associated with their greater need for precise control. If mild irregularities are observed on the medial surface of the musculomembranous vocal folds, high-pitched, low-pressure vocal gestures during stroboscopy will better delineate the geographic location of the subepithelial fibrosis. Given the precision requirements of classical performance, it is not surprising that nodules in opera singers were the most common lesions requiring surgery.

In the aforementioned model, the volume and pliability of the residual SLP are as important to vocal function as the lesion that is visualized on the surface. Stroboscopy is critical for voice management, since it is our principal method to assess the primary oscillator, the residual SLP. This functional model of vocal fold vibration is catalyzing future imaging methods such as confocal microscopy (unpublished data) and optical coherence tomography,^{49,50} which should provide improved methods to enhance evaluation of the SLP and associated lesions.

CONCEPTS IN PHONOMICROSURGERY: PAST, PRESENT, AND FUTURE

Repairing tissue trauma will remain in the domain of the surgeon for the foreseeable future, and there are probably very few singers without traumatic anatomic changes of the layered microstructure of their vocal folds. Essentially, singers are vocal athletes,

and it is normal for them to sustain performance-related soft tissue trauma. Therefore, the decision to perform surgery is based on whether the procedure to correct the trauma is less of a vocal liability than the self-induced soft tissue changes.

Until now, emphasis has been almost universally placed on the lesion, since our endolaryngeal resection techniques evolved from tumor surgery. In the 19th century, it consisted of piecemeal mirror-guided removal, and in the 20th century, it was done under direct vision and eventually with magnification. However, amputation with resection of the overlying epithelium without identifying the interface of the lesion base and the residual normal SLP often leads to variable results. The subepithelial resection approach^{28,42} is based on high-magnification dissection between the lesion and its surrounding normal tissue, the underlying SLP and overlying epithelium. Preservation of the epithelium precludes the need for secondary epithelialization, thus leading to faster healing. It is similar to raising a skin flap to remove a neck mass. We believe that the lack of epithelial preservation is a primary reason that investigations that compare amputation (similar to creating a superficial abrasion) by means of cold instruments to use of the CO₂ laser fail to find a substantial difference in function.^{51,52}

The volume of sublesional normal SLP is as significant to vocal function as the presence of a surface lesion and will therefore dictate future developments in endolaryngeal vocal edge surgery. Phonomicrosurgical procedures will evolve until it is commonplace to infuse^{20,22,38} an inert viscoelastic biomaterial or tissue-engineered SLP before a lesion resection. Gray et al and others have been working steadily to characterize the SLP biochemically and biomechanically.⁵³⁻⁵⁵ Insertion of a viscoelastic substance may even preclude the need for a resection of selected lesions if the basement membrane zone lesion is small. By simply infusing a biosubstance, we may be able to emulate the vibratory function of the musical-theater vocal fold. In future phonomicrosurgical procedures, placement of an SLP substitute in the vocal fold will become just as important as what is resected from the surface. This will enable us to treat vocal folds that have become stiff and nonpliable from long-term vocal use. This condition is often associated with the aging voice.

Since tissue engineering initiatives⁵⁶ should provide a pliable SLP substitute in the not-so-distant future, the number of candidates for surgical intervention should expand dramatically in the coming years. We believe that replacing lost SLP will reveal that many of the characteristics of the aging voice, especially in the vocalist, are the result of long-term trauma

to the SLP rather than true senescence. Since the most complex aspect of vocal performance is central nervous system processing, which remains largely undisturbed through most vocal careers, surgically repairing the lost pliability of the vocal folds will likely result in true vocal rejuvenation. Given the complexities and expectations of corrective voice surgery in singers, as well as the considerable attention that accompanies various performers, these surgical initiatives will probably serve as a harbinger of vocal edge surgery in the rest of the population. The exposure of these cases will probably catalyze new management standards for the rest of society, since the majority of hoarseness is the result of traumatically induced dysfunction in the SLP.

An increased frequency of procedures in the general population will result from the fact that most forms of employment are communication-based and many individuals are delaying retirement and desire to work later in life. There is even the potential for the incidence of phonotraumatic problems to increase with further development and application of computer voice recognition technology, especially as it replaces manual inputting (typing) of information.^{57,58}

Insufficient pliable SLP with associated dysphonia is a widespread problem in divergent patient populations. It may occur from any form of glottal instrumentation, including intubation (ie, premature infants). The population of successful laryngotracheal reconstruction patients typically exhibits limitations of vocal function,⁵⁹⁻⁶¹ primarily due to lost SLP, despite the fact that they may also have glottal closure deficits. Aerodynamic valving incompetence can now be corrected in a variety of circumstances by the extrapolation of Isshiki's¹⁵ framework procedures. Healed endoscopic glottal cancer defects represent a soft tissue model similar to the laryngotracheal reconstruction population in terms of vibratory insufficiency. Recent reconstructive initiatives⁶² can solve the aerodynamic leakage deficits of these children as they grow older. An SLP substitute may even provide the opportunity to treat poorly oscillating laryngeal tissue that results from irradiation.⁶³ Therefore, it is evident that the development of a biosubstitute for inadequate residual SLP in the musculo-membranous region remains the essential common pathway for voice restoration in these divergent populations.

PERFORMERS' ATTITUDES ABOUT SURGERY

Some performers, especially classical vocalists, are concerned that phonomicrosurgical intervention implies that they are singing incorrectly and therefore suggests compromised professionalism. We believe that this philosophy is incorrect and leads to undue

fears about future recovery and/or reinjury. Similar to other athletes, vocal performers should be perceived as professionals with potentially long careers for whom it is common to sustain injuries. Injuries in other athletic endeavors are not typically perceived as a limitation of skill or competency. Since high-level performance inherently involves the application of intense shearing stresses and collision forces to the delicate layered microstructure of the vocal folds, traumatic injury, to some degree, *should be expected*. In fact, many performers sustain injuries due to the magnitude and array of performance opportunities that arise from their success, as well as injuries due to their professionalism and desire to perform at their highest capabilities despite routine frailties such as the common cold. Singers are frequently performing with a repairable mechanical deficit, and phonomicrosurgical intervention can provide an opportunity for substantial career advancement.

It would be helpful if the stigma could be dispelled that compromised and unreliable performance is often associated with singers who require surgery. Toward that end, an open dialogue among performers as is consistent with issues of privacy would be beneficial to many vocalists past, present, and future. It is hoped that well-known experienced singers representing the spectrum of performance styles will take a leadership role in this issue, which would likely alleviate the anxiety of many young vocalists. Discussions of circumstances that predispose to injury might even provide constructive pathways to diminish performance-related phonotrauma. Eventually, this approach might alter practices of performance that are potentially unhealthy, ie, 5-show weekends and 8-show weeks (intramunicipal) and overscheduling of concert tours.

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GENERAL COMMENTS

The success encountered in this series is the result of a number of factors. The management of performing artists requires a collaborative team approach, in and out of the operating room. The speech pathologist and/or singing therapist is an especially critical member of the team. Phonomicrosurgery on performing artists should be done primarily under the highest possible magnification. The technique of epithelial cordotomy and subepithelial resection^{28,41,42} results in maximal preservation of the vocal folds' layered microstructure. This approach was designed to shorten healing time so performers could return to full activity as soon as possible. Typically, patients commence singing at 3 weeks and can be professionally performing in 5 to 8 weeks.

CONCLUSIONS

1. Successful phonomicrosurgical resection of most commonly encountered lesions can be achieved in singers and performing artists.
2. The success reported herein is the result of improvements in our understanding of the physiology underlying vocal fold oscillation combined with technical developments in phonomicrosurgical methods and instruments.
3. A team approach including sophisticated vocal rehabilitation is of paramount importance.
4. The patient and the surgeon must approach phonomicrosurgery collaboratively so that there is mutual responsibility for the decision to operate.
5. Vocal nodules can be treated successfully with phonomicrosurgery as part of a comprehensive management protocol.

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